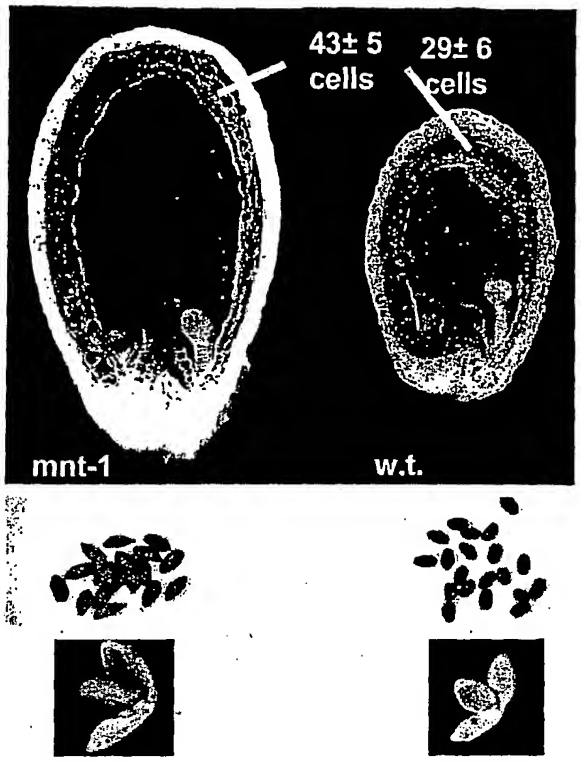
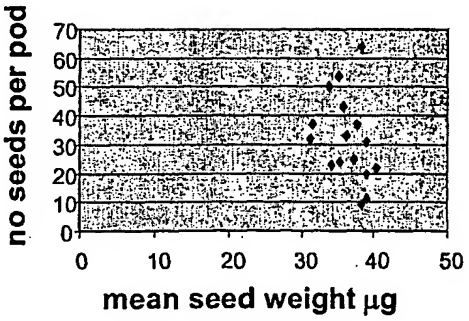
Figure 1

1A mnt-1 vs wild-type seeds



1B Seed weight vs no. seeds per pod in mnt-1



1C Maternal effect of mnt-1 mutation

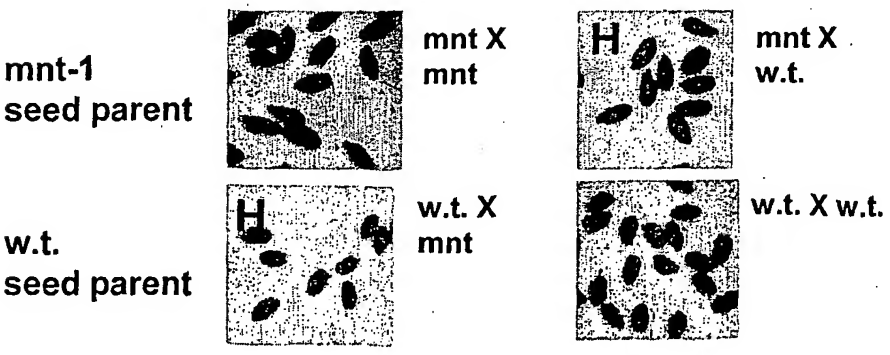
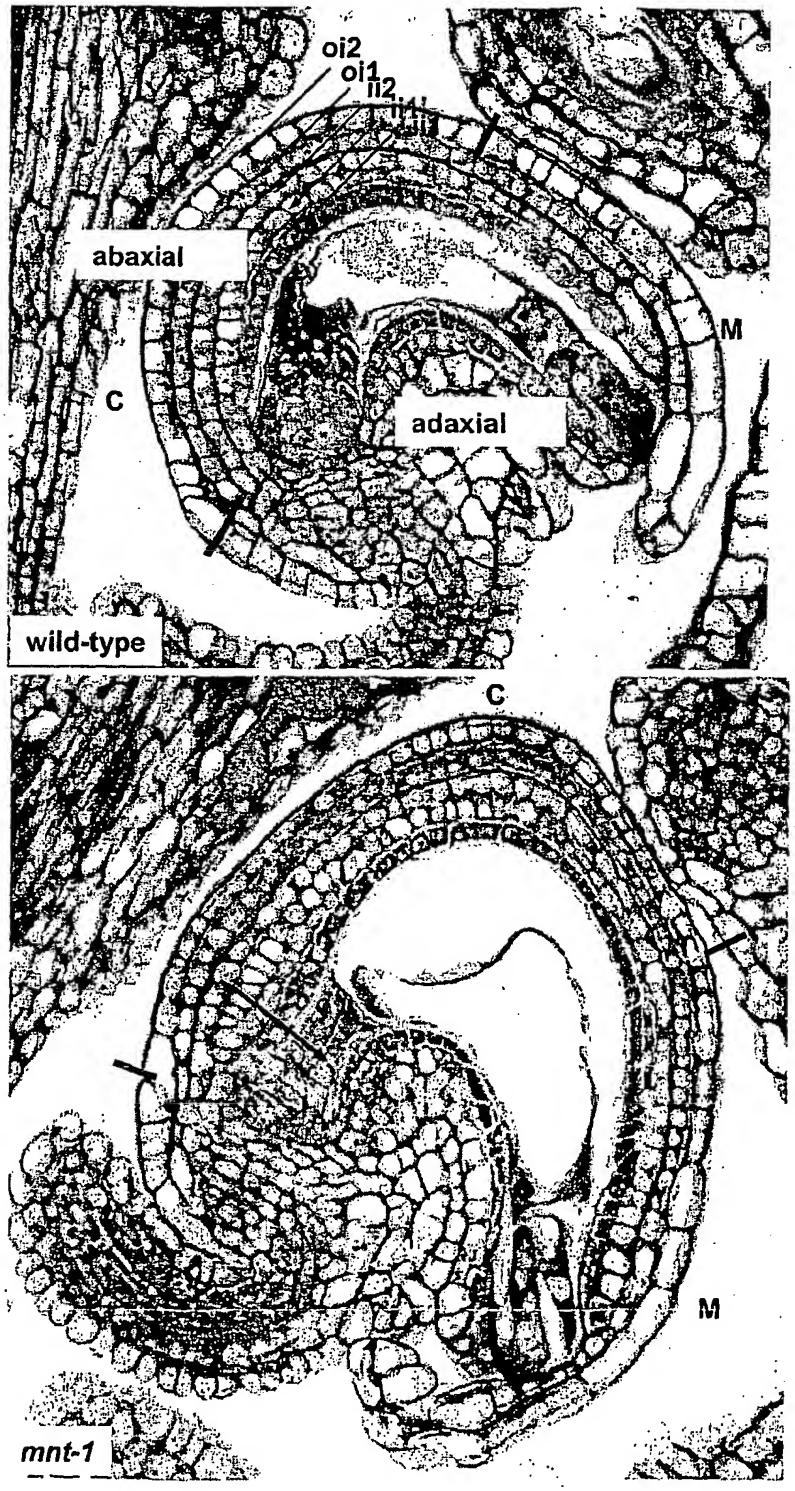


Figure 2

2A Mature w.t. and mnt-1 ovules



2B Cell number and size in w.t. and mnt-1 integuments

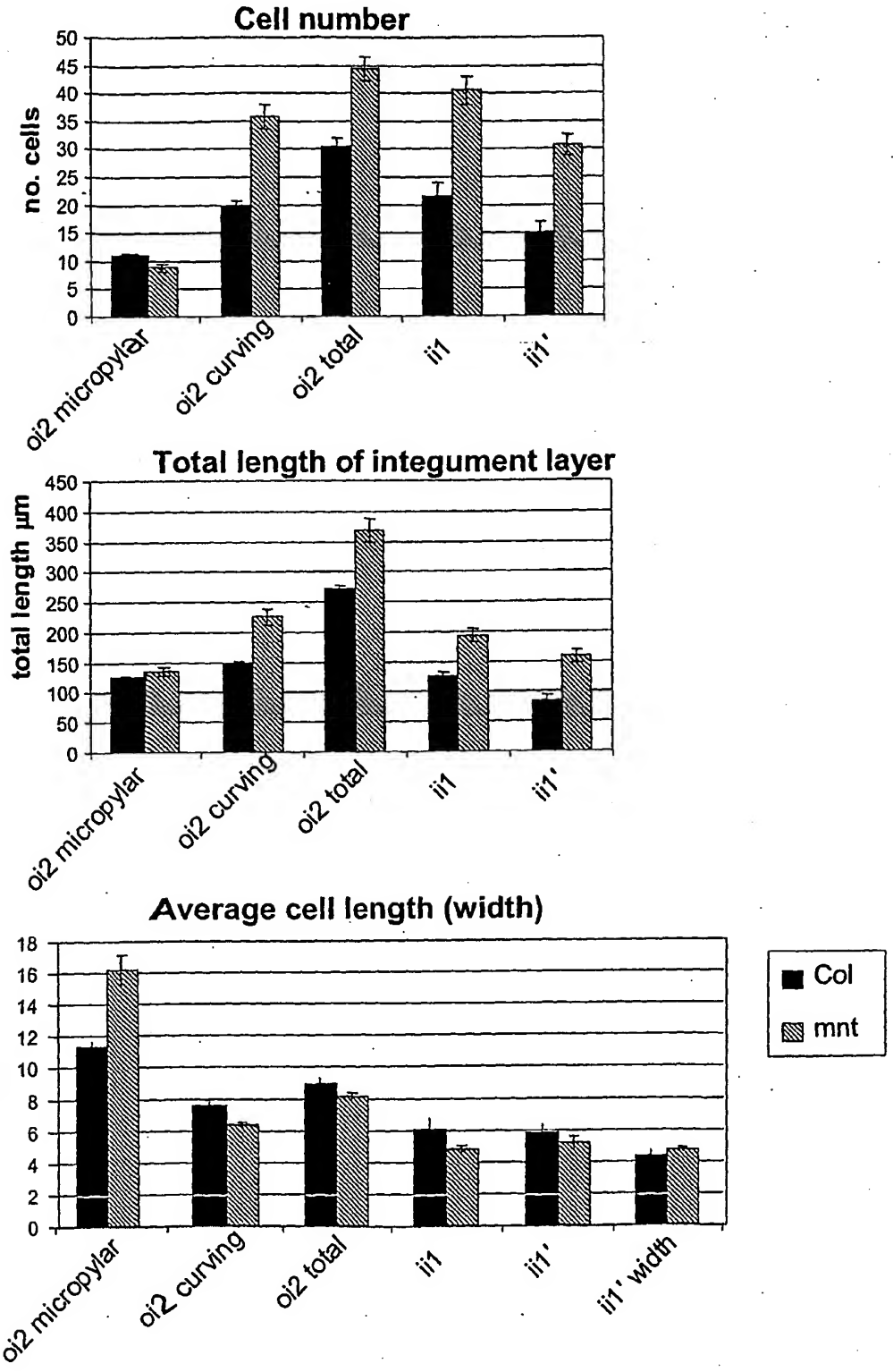
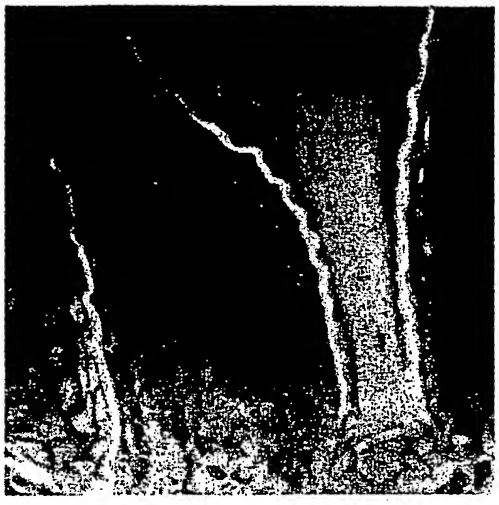


Figure 3
Chalazal endosperm

w.t. 7DAP



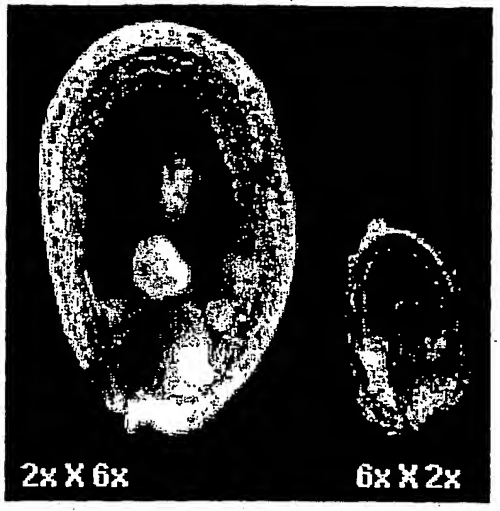
mnt-1 7DAP

2x X 6x 5 DAP

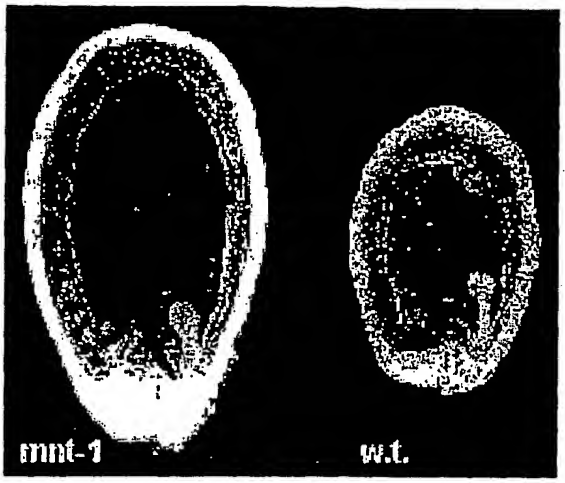
Bars = 50 μm

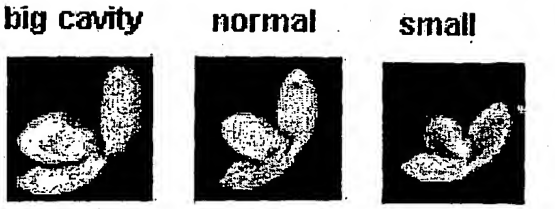
Figure 4

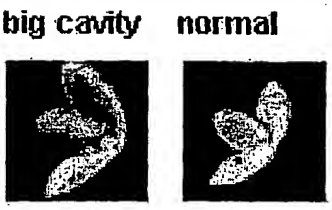
4A Endosperm-led growth

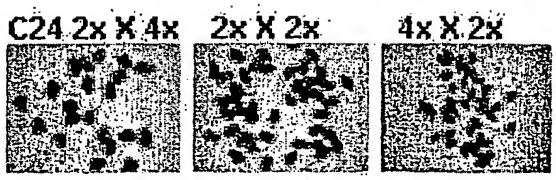


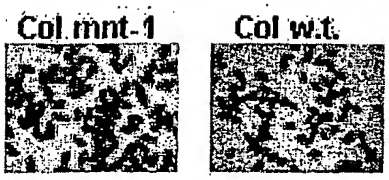
4B Integument-led growth











4C 'Big bag' hypothesis: seed and embyro size set by size of the seed cavity

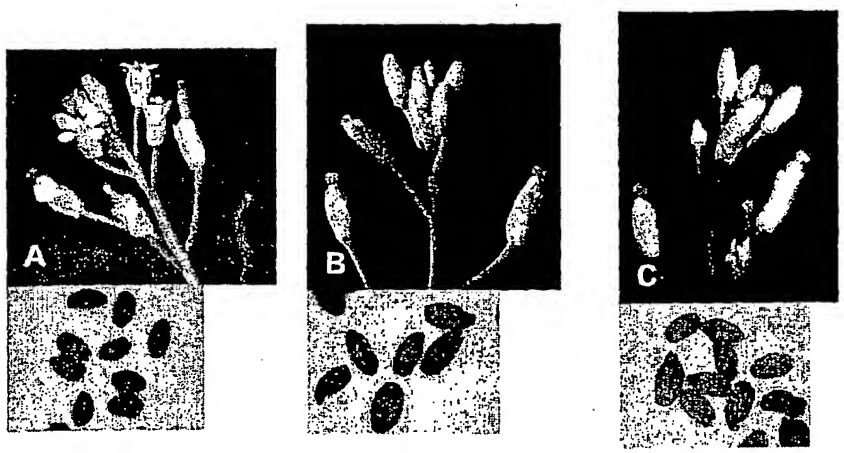
1. Division in endosperm (maternal and paternal control)

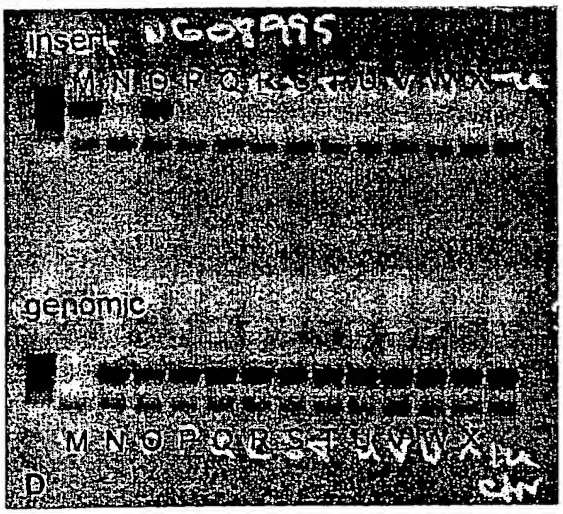
2. Division in integuments/ seed coat (maternal control)

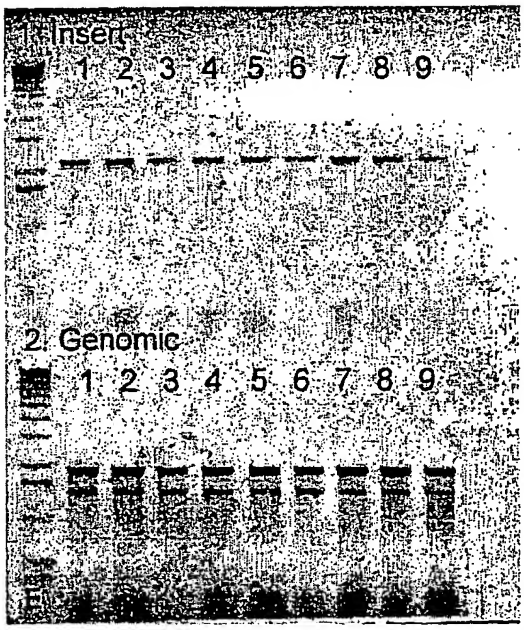
Figure 5

Allelism of mnt-1 and Salk insertion line 108995

Col-3 w.t. mnt-1 Salk 108995 homozygote







F1 mnt-1 X Salk 108995

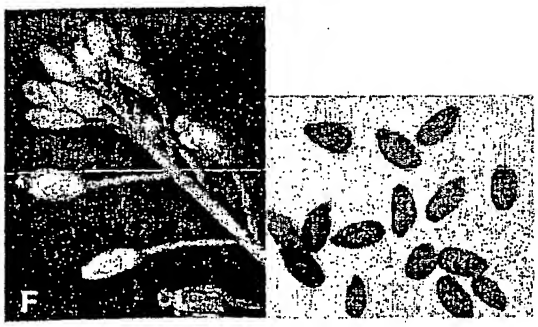
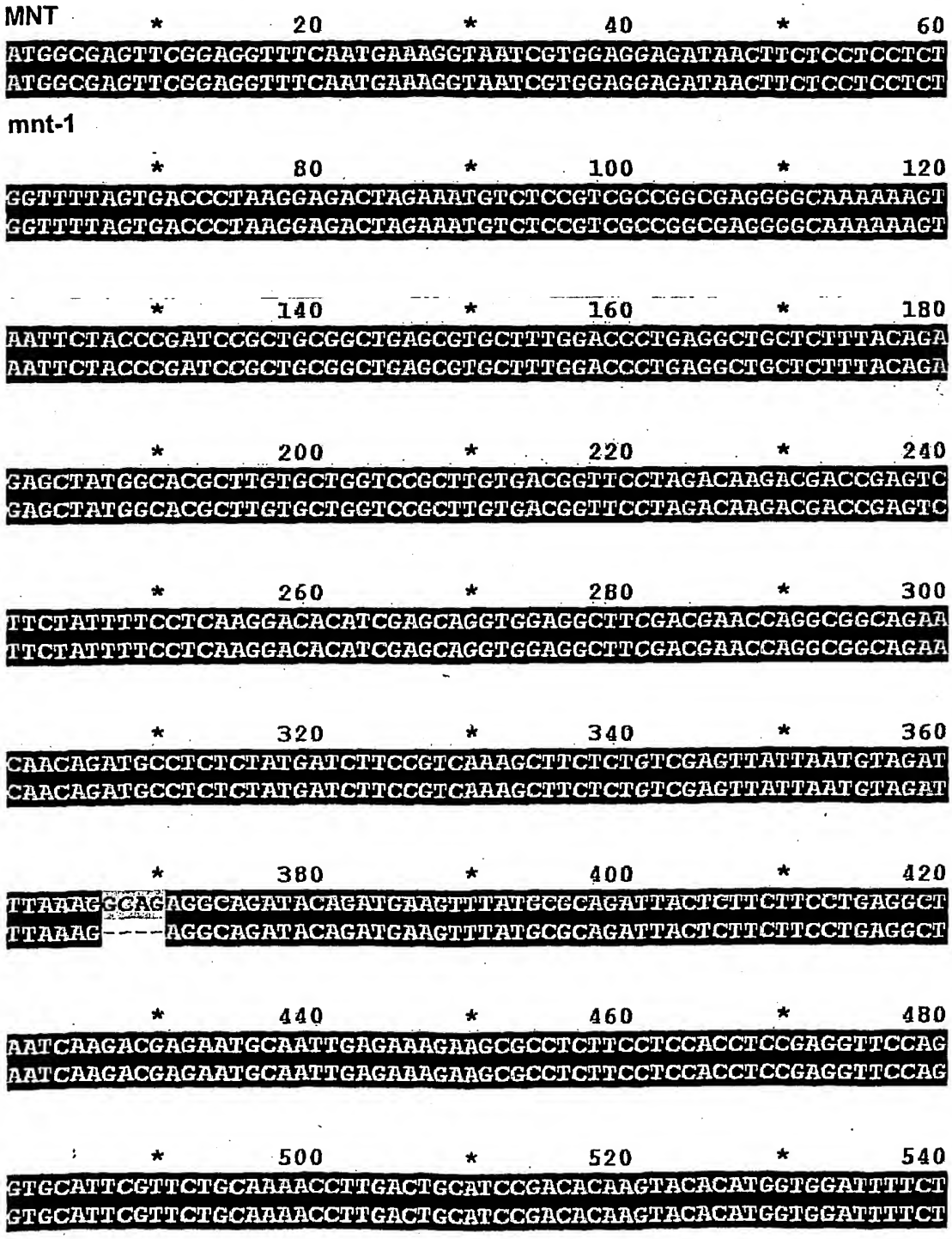


Figure 6

Alignment of w.t. MNT and mutant mnt-1 cDNA



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GTTCTTAGGCGACATGCGGATGAATGTCTCCCACCTCTGGATATGTCTCGACAGCCTCCC

        *       620         *       640         *       660
ACTCAAGAGTTAGTTGCAAAGGATTTGCATGCAAATGAGTGGCGATTCAGACATATATTC
ACTCAAGAGTTAGTTGCAAAGGATTTGCATGCAAATGAGTGGCGATTCAGACATATATTC

        *       680         *       700         *       720
CGGGGTCAACCACGGAGGCATTTGCTACAGAGTGGGTGGAGTGTGTTTGTTAGCTCCAAA
CGGGGTCAACCACGGAGGCATTTGCTACAGAGTGGGTGGAGTGTGTTTGTTAGCTCCAAA

        *       740         *       760         *       780
AGGCTAGTTGCAGGCGATGCGTTTATATTTCTAAGGGGCGAGAATGGAGAATTAAGAGTT
AGGCTAGTTGCAGGCGATGCGTTTATATTTCTAAGGGGCGAGAATGGAGAATTAAGAGTT

        *       800         *       820         *       840
GGTGTAAGGCGTGCGATGCGACAACAAGGAAACGTGCCGTCTTCTGTTATATCTAGCCAT
GGTGTAAGGCGTGCGATGCGACAACAAGGAAACGTGCCGTCTTCTGTTATATCTAGCCAT

        *       860         *       880         *       900
AGCATGCATCTTGGAGTACTGGCCACCGCATGGCATGCCATTTCAACAGGGACTATGTTT
AGCATGCATCTTGGAGTACTGGCCACCGCATGGCATGCCATTTCAACAGGGACTATGTTT

        *       920         *       940         *       960
ACAGTCTACTACAAACCCAGGACGAGCCCATCTGAGTTTATTGTTCCGTTCGATCAGTAT
ACAGTCTACTACAAACCCAGGACGAGCCCATCTGAGTTTATTGTTCCGTTCGATCAGTAT

        *       980         *       1000        *       1020
ATGGAGTCTGTTAAGAATAACTACTCTATTGGCATGAGATTCAAAATGAGATTTGAAGGC
ATGGAGTCTGTTAAGAATAACTACTCTATTGGCATGAGATTCAAAATGAGATTTGAAGGC

        *       1040        *       1060        *       1080
GAAGAGGCTCCTGAGCAGAGGTTTACTGGCACAATCGTTGGGATTGAAGAGTCTGATCCT
GAAGAGGCTCCTGAGCAGAGGTTTACTGGCACAATCGTTGGGATTGAAGAGTCTGATCCT

        *       1100        *       1120        *       1140
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ACTAGGTGGCCAAAATCAAAGTGGAGATCCCTCAAGGTGAGATGGGATGAGACTTCTAGT
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          *       1160          *       1180          *       1200
ATTCCTCGACCTGATAGAGTATCTCCGTGGAAAGTAGAGCCAGCTCTTGCTCCTCCTGCT
ATTCCTCGACCTGATAGAGTATCTCCGTGGAAAGTAGAGCCAGCTCTTGCTCCTCCTGCT

          *       1220          *       1240          *       1260
TTGAGTCCTGTTCCAATGCCTAGGCCTAAGAGGCCCAGATCAAATATAGCACCTTCATCT
TTGAGTCCTGTTCCAATGCCTAGGCCTAAGAGGCCCAGATCAAATATAGCACCTTCATCT

          *       1280          *       1300          *       1320
CCTGACTCTTCGATGCTTACCAGAGAAGGTACAACTAAGGCAAACATGGACCCTTTACCA
CCTGACTCTTCGATGCTTACCAGAGAAGGTACAACTAAGGCAAACATGGACCCTTTACCA

          *       1340          *       1360          *       1380
GCAAGCGGACTTTCAAGGGTCTTGCAAGGTCAAGAATACTCGACCTTGAGGACGAAACAT
GCAAGCGGACTTTCAAGGGTCTTGCAAGGTCAAGAATACTCGACCTTGAGGACGAAACAT

          *       1400          *       1420          *       1440
ACTGAGAGTGTAGAGTGTGATGCTCCTGAGAATTCTGTTGTCTGGCAATCTTCAGCGGAT
ACTGAGAGTGTAGAGTGTGATGCTCCTGAGAATTCTGTTGTCTGGCAATCTTCAGCGGAT

          *       1460          *       1480          *       1500
GATGATAAGGTTGACGTGGTTTCGGGTTCTAGAAGATATGGATCTGAGAACTGGATGTCC
GATGATAAGGTTGACGTGGTTTCGGGTTCTAGAAGATATGGATCTGAGAACTGGATGTCC

          *       1520          *       1540          *       1560
TCAGCCAGGCATGAACCTACTTACACAGATTTGCTCTCCGGCTTTGGGACTAACATAGAT
TCAGCCAGGCATGAACCTACTTACACAGATTTGCTCTCCGGCTTTGGGACTAACATAGAT

          *       1580          *       1600          *       1620
CCATCCCATGGTCAGCGGATACCTTTTTTATGACCATTCATCATCACCTTCTATGCCTGCA
CCATCCCATGGTCAGCGGATACCTTTTTTATGACCATTCATCATCACCTTCTATGCCTGCA

          *       1640          *       1660          *       1680
AAGAGAATCTTGAGTGATTCAGAAGGCAAGTTCGATTATCTTGCTAACCAGTGGCAGATG
AAGAGAATCTTGAGTGATTCAGAAGGCAAGTTCGATTATCTTGCTAACCAGTGGCAGATG

          *       1700          *       1720          *       1740
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ATACACTCTGGTCTCTCCCTGAAGTTACATGAATCTCCTAAGGTACCTGCAGCAACTGAT
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          *       1760          *       1780          *       1800
GCGTCTCTCCAAGGGCGATGCAATGTTAAATACAGCGAATATCCTGTTCTTAATGGTCTA
GCGTCTCTCCAAGGGCGATGCAATGTTAAATACAGCGAATATCCTGTTCTTAATGGTCTA

          *       1820          *       1840          *       1860
TCGACTGAGAATGCTGGTGGTAACTGGCCAATACGTCCACGTGCTTTGAATTATTATGAG
TCGACTGAGAATGCTGGTGGTAACTGGCCAATACGTCCACGTGCTTTGAATTATTATGAG

          *       1880          *       1900          *       1920
GAAGTGGTCAATGCTCAAGCGCAAGCTCAGGCTAGGGAGCAAGTAACAAAACAACCCTTC
GAAGTGGTCAATGCTCAAGCGCAAGCTCAGGCTAGGGAGCAAGTAACAAAACAACCCTTC

          *       1940          *       1960          *       1980
ACGATACAAGAGGAGACAGCAAAGTCAAGAGAAGGGAACTGCAGGCTCTTTGGCATTCCT
ACGATACAAGAGGAGACAGCAAAGTCAAGAGAAGGGAACTGCAGGCTCTTTGGCATTCCT

          *       2000          *       2020          *       2040
CTGACCAACAACATGAATGGGACAGACTCAACCATGTCTCAGAGAAACAACTTGAATGAT
CTGACCAACAACATGAATGGGACAGACTCAACCATGTCTCAGAGAAACAACTTGAATGAT

          *       2060          *       2080          *       2100
GCTGCGGGGCTTACACAGATAGCATCACCAAAGGTTCAGGACCTTTCAGATCAGTCAAAA
GCTGCGGGGCTTACACAGATAGCATCACCAAAGGTTCAGGACCTTTCAGATCAGTCAAAA

          *       2120          *       2140          *       2160
GGGTCAAAAATCAACAAACGATCATCGTGAACAGGGAAGACCATTCCAGACTAATAATCCT
GGGTCAAAAATCAACAAACGATCATCGTGAACAGGGAAGACCATTCCAGACTAATAATCCT

          *       2180          *       2200          *       2220
CATCCGAAGGATGCTCAAACGAAAACCAACTCAAGTAGGAGTTGCACAAAGGTTCACAAG
CATCCGAAGGATGCTCAAACGAAAACCAACTCAAGTAGGAGTTGCACAAAGGTTCACAAG

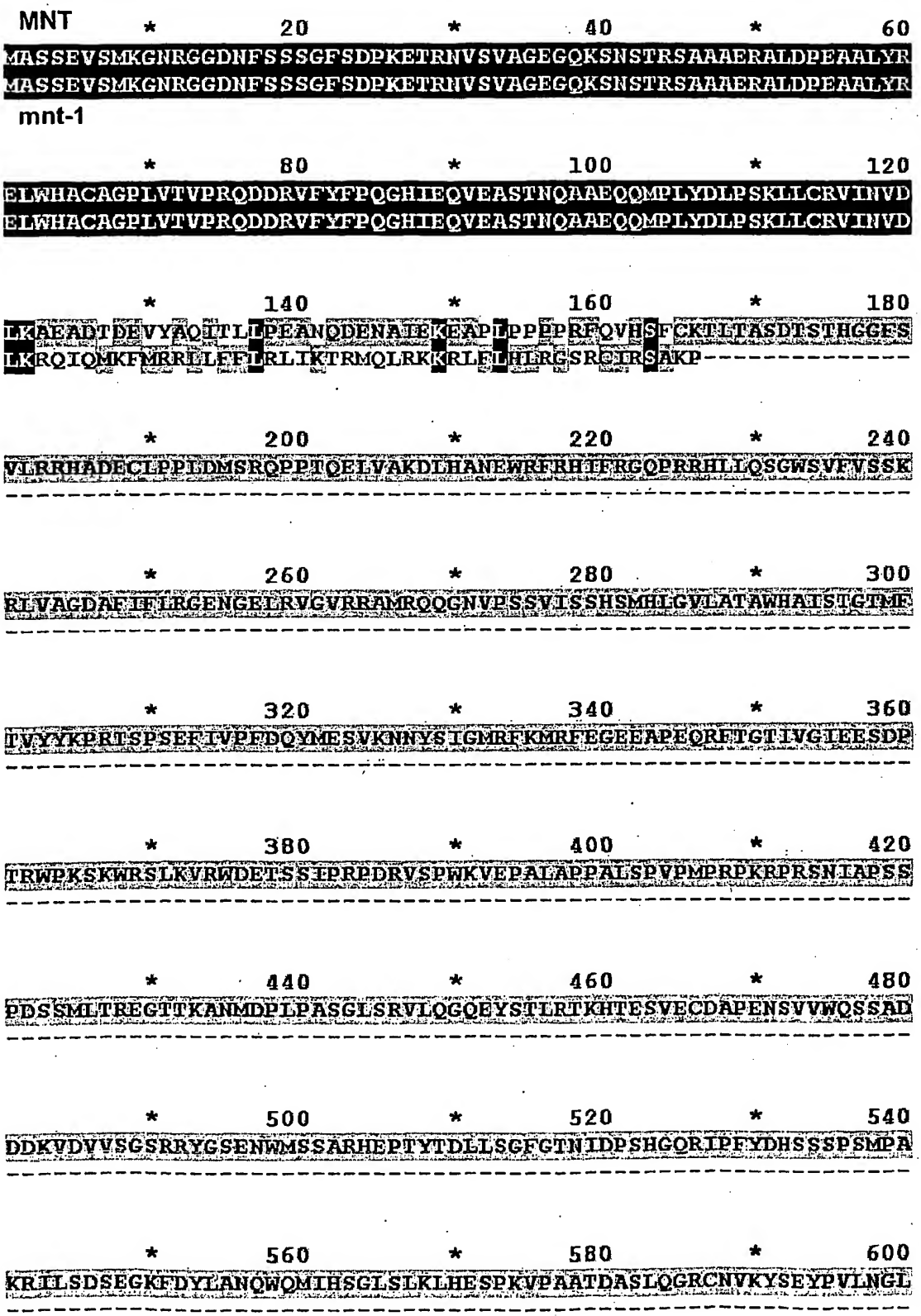
          *       2240          *       2260          *       2280
CAGGGAATTGCACTTGGCCGTTCAGTGGATCTTTCAAAGTTCCAAAACTATGAGGAGTTA
CAGGGAATTGCACTTGGCCGTTCAGTGGATCTTTCAAAGTTCCAAAACTATGAGGAGTTA

          *       2300          *       2320          *       2340
GTCGCTGAGCTGGACAGGCTGTTTGAGTTCAATGGAGAGTTGATGGCTCCTAAGAAAGAT
GTCGCTGAGCTGGACAGGCTGTTTGAGTTCAATGGAGAGTTGATGGCTCCTAAGAAAGAT
```

* 2360 * 2380 * 2400
TGGTTGATAGTTTACACAGATGAAGAGAATGATATGATGCTTGTTGGTGACGATCCTTGG
TGGTTGATAGTTTACACAGATGAAGAGAATGATATGATGCTTGTTGGTGACGATCCTTGG
* 2420 * 2440 * 2460
CAGGAGTTTTGTTGCATGGTTCGCAAAATCTTCATATACACGAAAGAGGAAGTGAGGAAG
CAGGAGTTTTGTTGCATGGTTCGCAAAATCTTCATATACACGAAAGAGGAAGTGAGGAAG
* 2480 * 2500 * 2520
ATGAACCCGGGGACTTTAAGCTGTAGGAGCGAGGAAGAAGCAGTTGTTGGGGAAGGATCA
ATGAACCCGGGGACTTTAAGCTGTAGGAGCGAGGAAGAAGCAGTTGTTGGGGAAGGATCA
* 2540 * 2560 * 2580
GATGCAAAGGACGCCAAGTCTGCATCAAATCCTTCATTGTCCAGCGCTGGGAACTCTTAA
GATGCAAAGGACGCCAAGTCTGCATCAAATCCTTCATTGTCCAGCGCTGGGAACTCTTAA

Figure 7

Alignment of w.t. MNT and mutant mnt-1 protein



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          *         620         *         640         *         660
STENAGGNWPIRPRALNYYEEVVNAQAQAQAREQVTKQPFTIQEETAKSREGNCRLFGIP
------------------------------------------------------------

          *         680         *         700         *         720
LTNNMNGTDSTMSQRNNLNDAAGLTQIASPKVQDLSDQSKGSKSTNDHREQGRPFQTNNP
------------------------------------------------------------

          *         740         *         760         *         780
HPKDAQTKTNSSRSCTKVHKQGIALGRSVDLSKFQNYEELVAELDRLFEFNGELMAPKKD
------------------------------------------------------------

          *         800         *         820         *         840
WLTVYTDEENDMMLVGDDPWQEFCCMVRKIFIYTKEEVRKMNPGTLSCRSEEEAVVGEGS
------------------------------------------------------------

          *
DAKDAKSASNPSLSSAGNS
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```
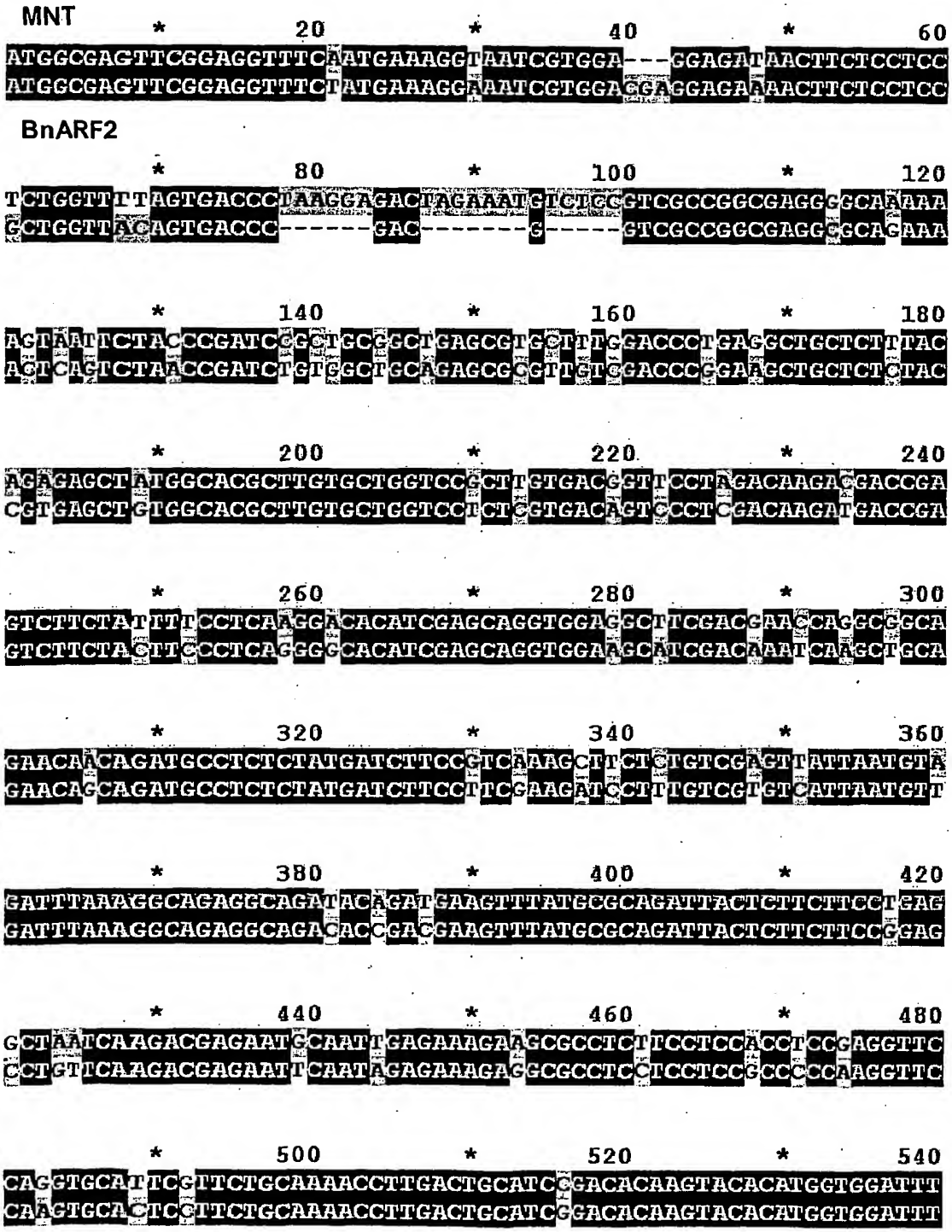
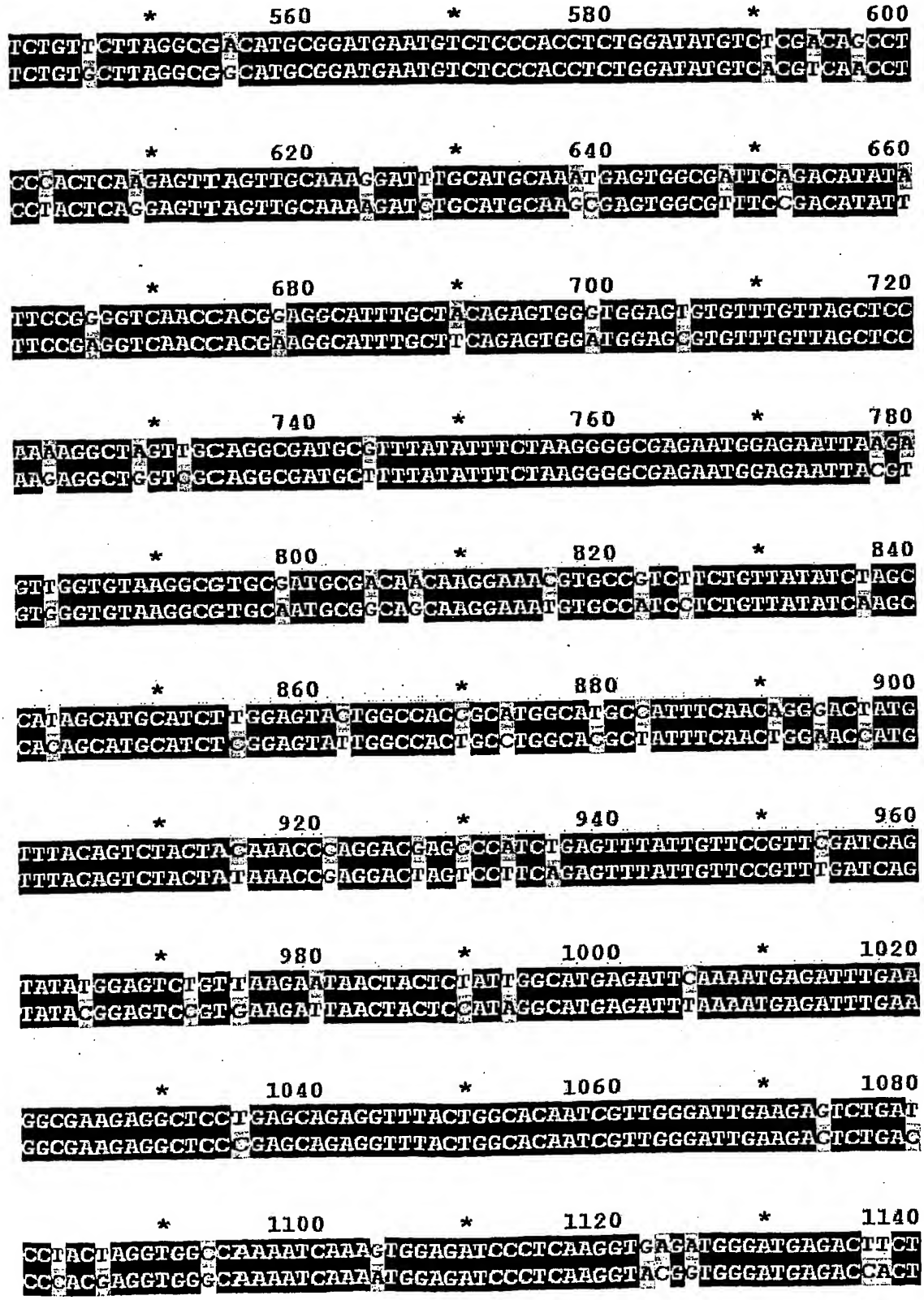

Figure 8

Alignment of MNT and BnARF2 cDNA

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680
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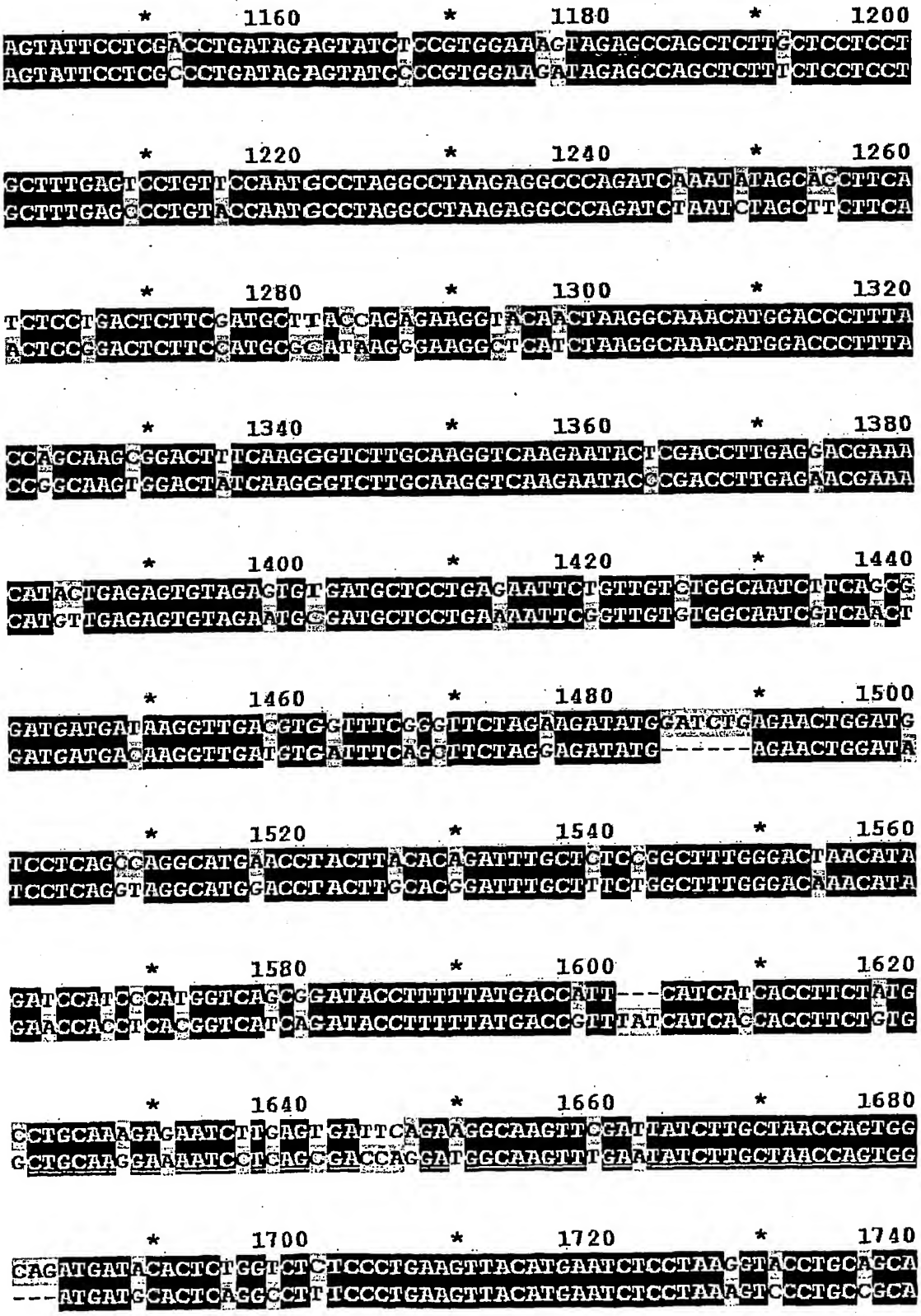
1100
1120
1140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1180
1200
1220
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1260
1280
1300
1320
1340
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1380
1400
1420
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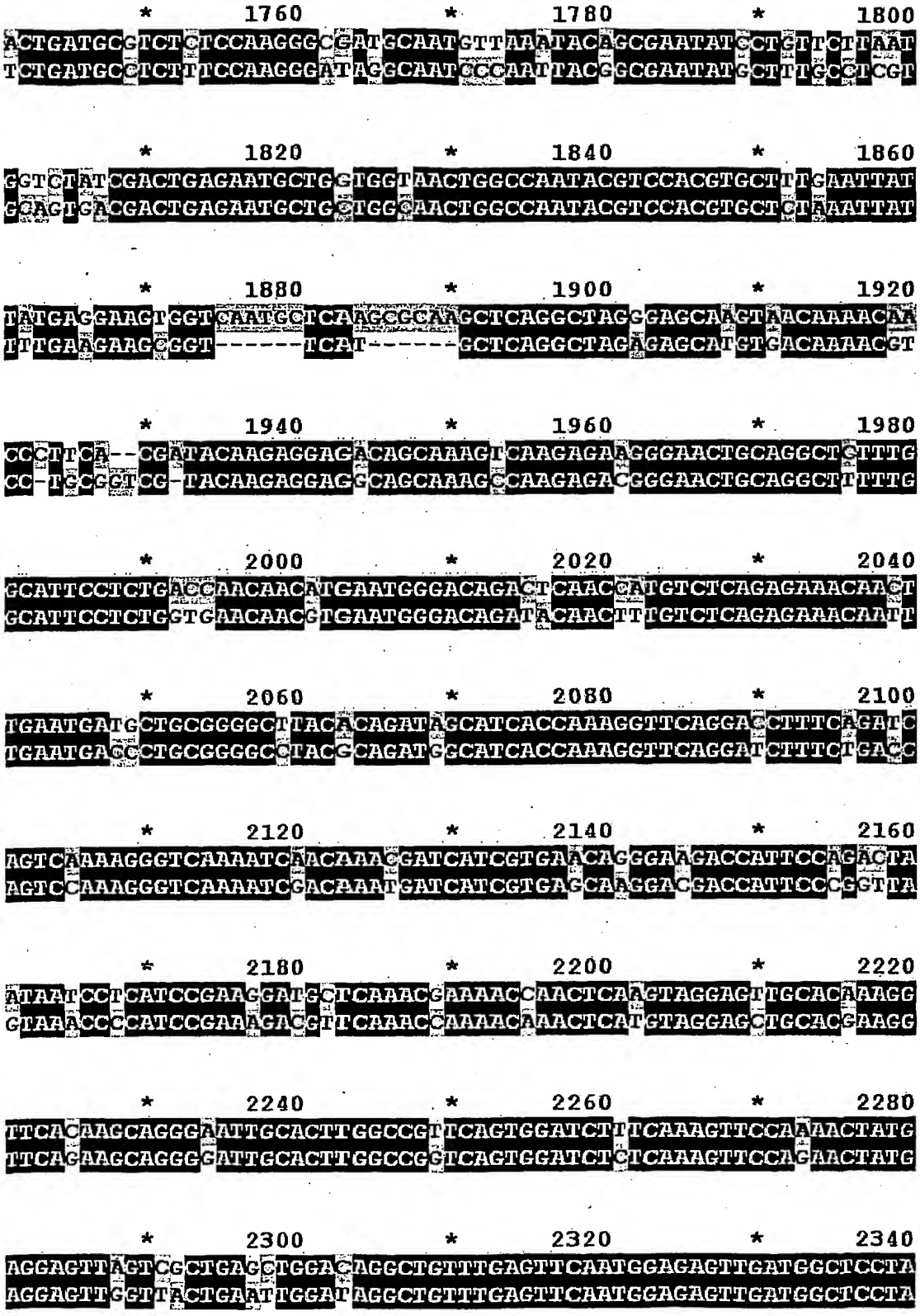
1700
1720
1740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TCTGATGCCTCTTTCCAAGGGATAGGCAATCCCAATTACGGCGAATATGCTTTGCCTCGT
* 1820 * 1840 * 1860
GGTCTATCGACTGAGAATGCTGGTGGTAACTGGCCAATACGTCCACGTGCTTTGAATTAT
GCAGTGACGACTGAGAATGCTGCTGGCAACTGGCCAATACGTCCACGTGCTCTAAATTAT
* 1880 * 1900 * 1920
TATGAGGAAGTGGTCAATGCTCAAGCGCAAGCTCAGGCTAGGGAGCAAGTAACAAAACAA
TTTGAAGAAGCGGT------TCAT------GCTCAGGCTAGAGAGCATGTGACAAAACGT
* 1940 * 1960 * 1980
CCCTTCA--CGATACAAGAGGAGACAGCAAAGTCAAGAGAAGGGAACTGCAGGCTGTTTG
CC-TGCGCTCG-TACAAGAGGAGGCAGCAAAGCCAAGAGACGGGAACTGCAGGCTTTTTG
* 2000 * 2020 * 2040
GCATTCCTCTGACCAACAACATGAATGGGACAGACTCAACGATGTCTCAGAGAAACAACT
GCATTCCTCTGGTGAACAACGTGAATGGGACAGATACAACTTTGTCTCAGAGAAACAATT
* 2060 * 2080 * 2100
TGAATGATGCTGCGGGGGCTTACACAGATAGCATCACCAAAGGTTCAGGAGCTTTCAGATC
TGAATGACGCTGCGGGGGCCTACGCAGATGGCATCACCAAAGGTTCAGGATCTTTCTGACC
* 2120 * 2140 * 2160
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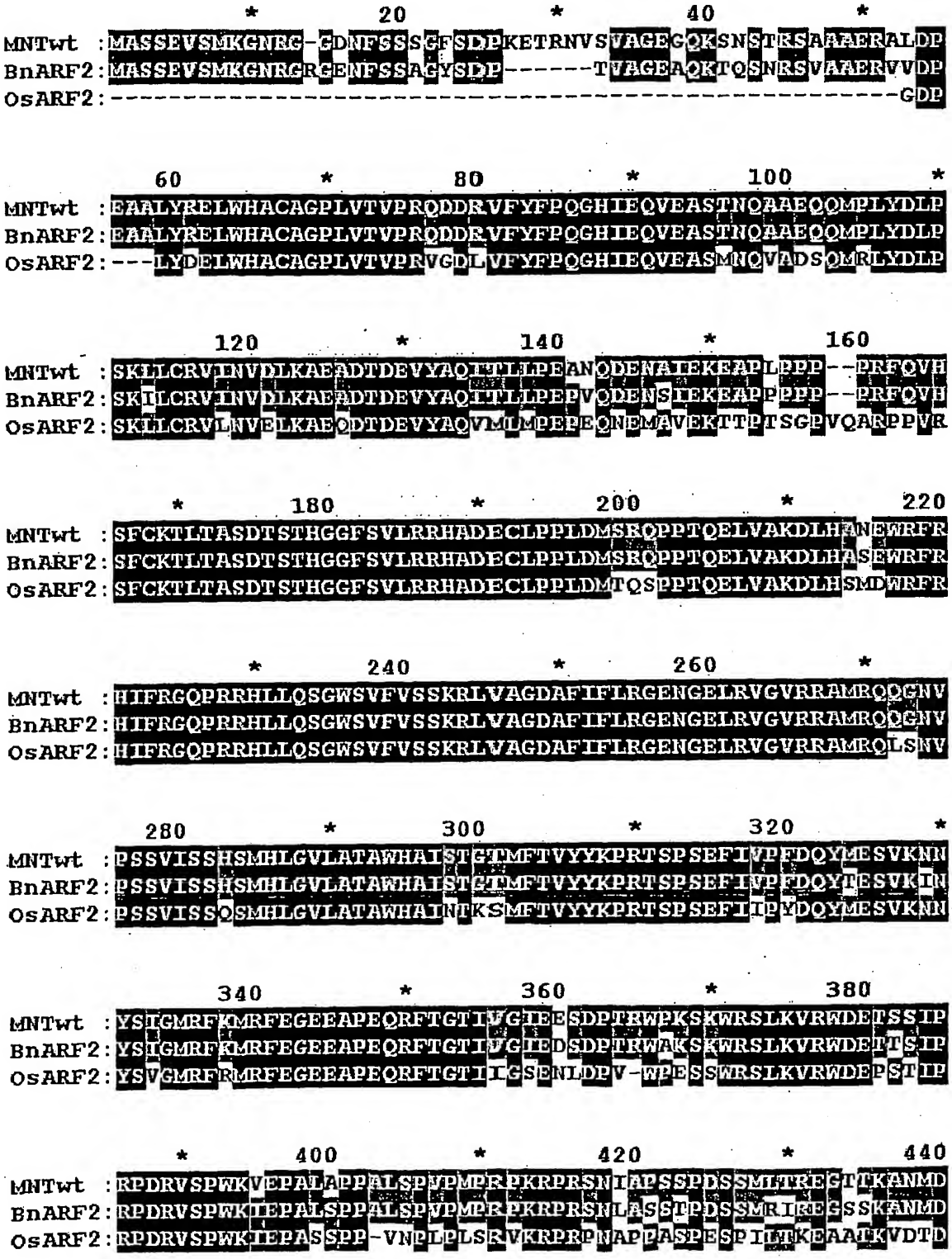
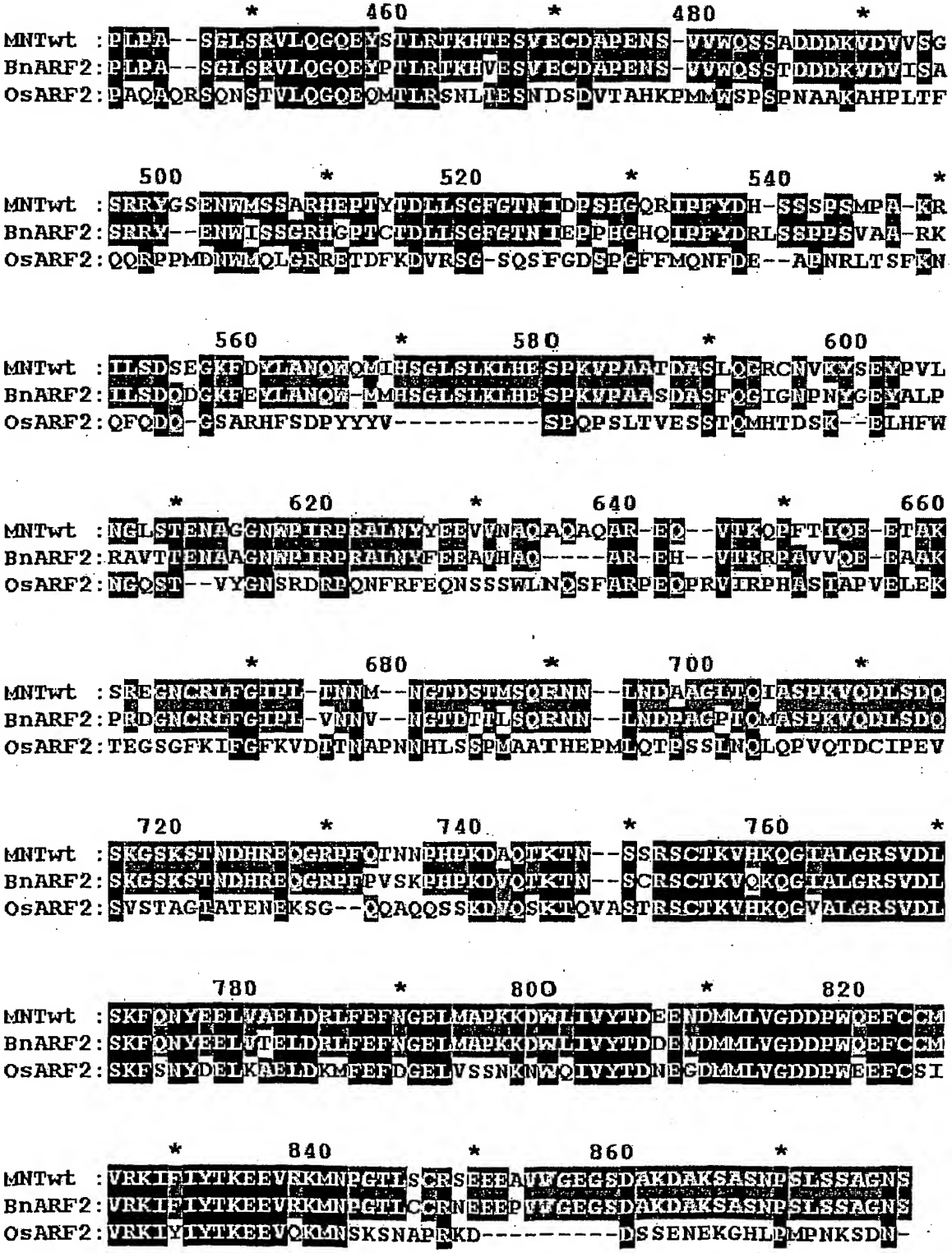
AGTCCAAAGGGTCAAAATCGACAAATGATCATCGTGAGCAAGGACGACCATTCCCGGTTA
* 2180 * 2200 * 2220
ATAATCCTCATCCGAAGGATGCTCAAACGAAAACCAACTCAAGTAGGAGTTGCACAAAGG
GTAAACCCCATCCGAAACACGTTCAAACCAAAACAAACTCATGTAGGAGCTGCACGAAGG
* 2240 * 2260 * 2280
TTCACAAGCAGGGAATTGCACTTGGCCGTTCAGTGGATCTTTCAAAGTTCCAAAACTATG
TTCAGAAGCAGGGGATTGCACTTGGCCGGTCAGTGGATCTCTCAAAGTTCCAGAACTATG
* 2300 * 2320 * 2340
AGGAGTTACTCGCTGAGCTGGACAGGCTGTTTGAGTTCAATGGAGAGTTGATGGCTCCTA
AGGAGTTGGTTACTGAATTGGATAGGCTGTTTGAGTTCAATGGAGAGTTGATGGCTCCTA

* 2360 * 2380 * 2400
AGAAAGATTGGTTGATAGTTTACACAGATGAAGAGAATGATATGATGCTTGTTGGTGACG
AGAAAGATTGGCTGATAGTTTACACAGATGATGAGAATGATATGATGCTTGTTGGAGACG
* 2420 * 2440 * 2460
ATCCTTGGCAGGAGTTTTGTTGCATGGTTCGCAAAATCTTCATATACACGAAAGAGGAAG
ATCCTTGGCAGGAGTTTTGTTGCATGGTTCGTAAAATCTTCATATACACGAAAGAGGAGG
* 2480 * 2500 * 2520
TGAGGAAGATGAACCCGGGGACTTTAAGCTGTAGGAGCGAGGAAGAAGCAGTTGTTGGGG
TGAGGAAGATGAACCCGGGAACTCTATGCTGTAGGAACGAGGAAGAACCAGTTGTTGGGG
* 2540 * 2560 * 2580
AAGGATCAGATGCAAAGGACGCCAAGTCTGCATCAAATCCTTCATTGTCCAGCGCTGGCA
AAGGATCAGATGCAAAGGACGCGAAGTCTGCATCAAATCCTTCATTGTCCAGCGCCGGAA
ACTCTTAA
ACTCTTAA

Figure 9

Alignment of MNT, BnARF2, OsARF2 proteins

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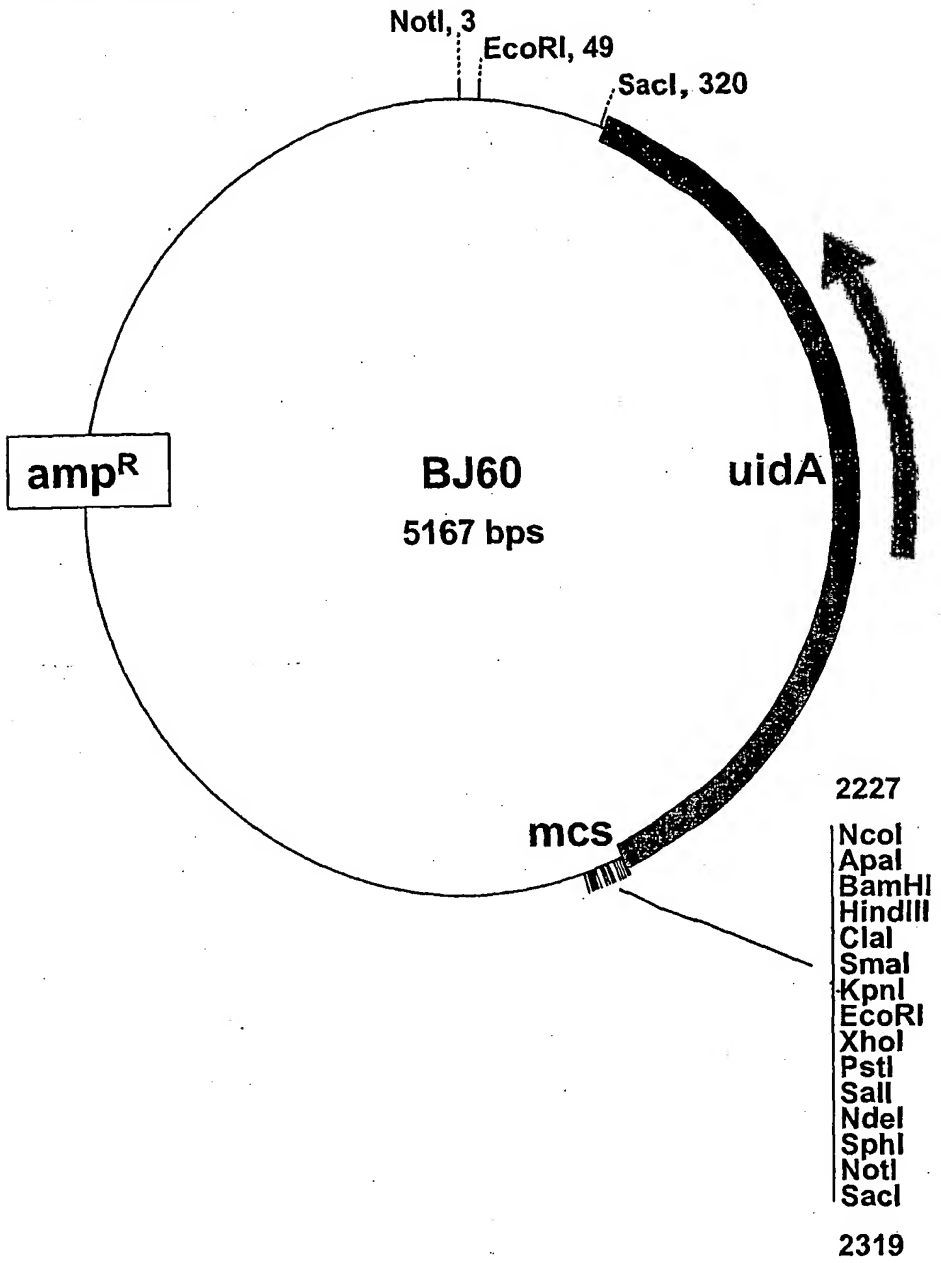
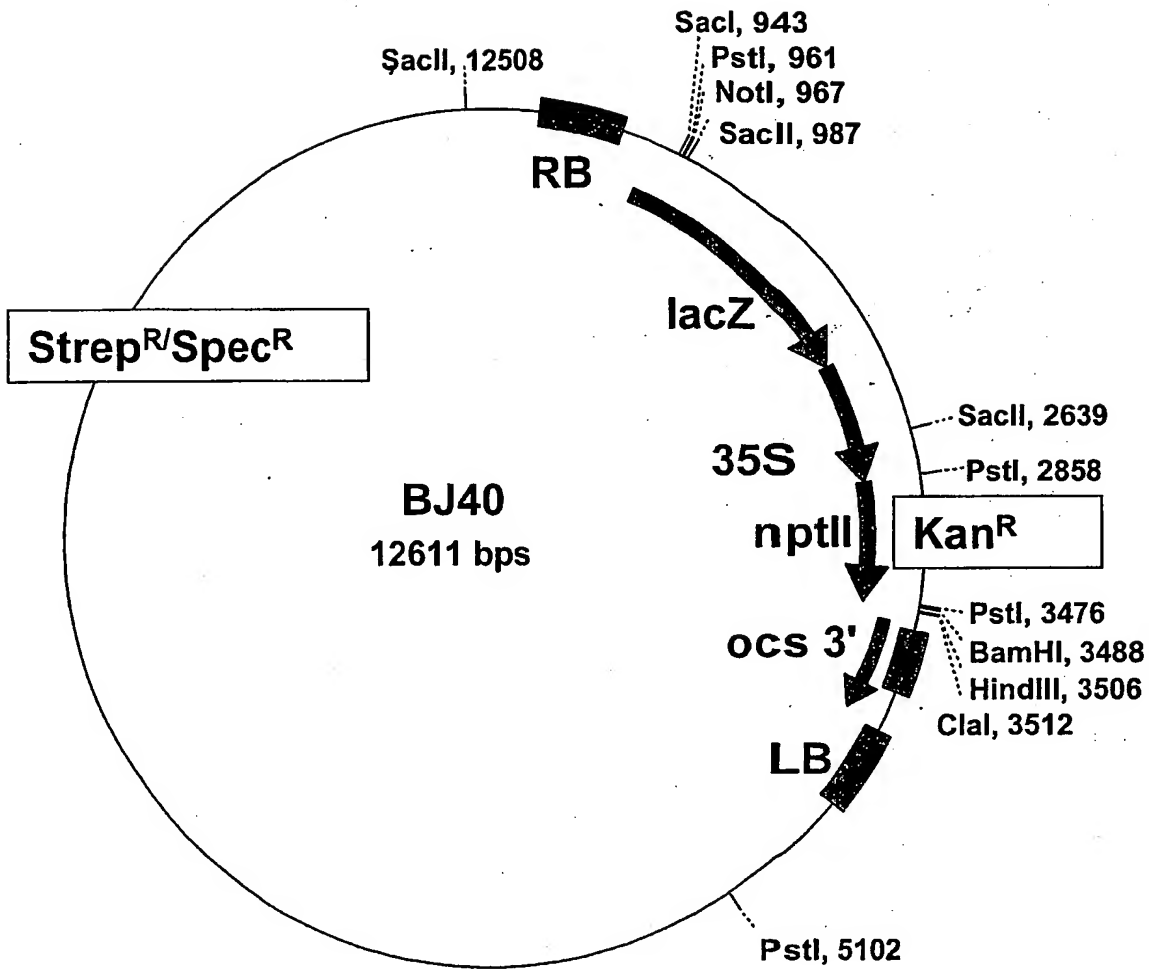
BnARF2:PLPA--SGLSRVLQGQEYPTLRTKHVESVECDAPENS-VVWQSSTDDDKVDVISA
OsARF2:PAQAQRSQNSTVLQGQEQMTLRSNLTESNDSDVTAHKPMMWSPSPNAAKAHPLTF
500 520 540
MNTwt :SRRYGSENWMSSARHEPTYTDLLSGFGTNIDPSHGQRIPFYDH-SSSPSMPA-KR
BnARF2:SRRY--ENWISSGRHGPTCTDLLSGFGTNIEPPHGHQIPFYDRLSSPPSVAA-RK
OsARF2:QQRPPMDNWMQLGRRETDFKDVRSG-SQSFGDSPGFFMQNFDE--APNRLTSFKN
560 580 600
MNTwt :ILSDSEGKFDYLANQWQMIHSGLSLKLHESPKVPAATDASLQGRCNVKYSEYPVL
BnARF2:ILSDQDGKFEYLANQW-MMHSGLSLKLHESPKVPAASDASFQGIGNPNYGEYALP
OsARF2:QFQDQ-GSARHFSDPYYYV----------SPQPSLTVESSTQMHTDSK--ELHFW
620 640 660
MNTwt :NGLSTENAGGNFPIRPRALNYYEEVVNAQAQAQARE-EQ--VTKQPFTIQE-ETAK
BnARF2:RAVTTENAAGNFPIRPRALNYFEEAVHAQ-----AR-EH--VTKRPAVVQE-EAAK
OsARF2:NGQST--VYGNSRDRPQNFRFEQNSSSWLNQSFARPEQPRVIRPHASIAPVELEK
680 700
MNTwt :SREGNGRLFGIPL-TNNM--NGTDSTMSQFNN--LNDAAGITQIASPKVQDLSDQ
BnARF2:PRDGNGRLFGIPL-VNNV--NGTDTTLSQRNN--LNDPAGPTQMASPKVQDLSDQ
OsARF2:TEGSGFKIFGFKVDITNAPNNHLSSPMAATHEPMLQTPSSLNQLQPVQTDCIPEV
720 740 760
MNTwt :SKGSKSTNDHREQGRPFQTNNPHPKDAQTKTN--SSRSCTKVHKQGIALGRSVDL
BnARF2:SKGSKSTNDHREQGRPFPVSKPHPKDVQTKTN--SCRSCTKVQKQGIALGRSVDL
OsARF2:SVSTAGTATENEKSG--QQAQQSSKDVQSKTQVASTRSCTKVHKQGVALGRSVDL
780 800 820
MNTwt :SKFQNYEELVAELDRLFEFNGELMAPKKDWLIVYTDEENDMMLVGDDPWQEFCCM
BnARF2:SKFQNYEELVTELDRLFEFNGELMAPKKDWLIVYTDDENDMMLVGDDPWQEFCCM
OsARF2:SKFSNYDELKAELDKMFEFDGELVSSNKNWQIVYTDNEGDMMLVGDDPWEEFCSI
840 860
MNTwt :VRKIFIYTKEEVRKMNPGTLSCRSEEEAVFGEGGSDAKDAKSASNPSLSSAGNS
BnARF2:VRKIFIYTKEEVRKMNPGTLCCRNEEEPWFGEGGSDAKDAKSASNPSLSSAGNS
OsARF2:VRKIYIYTKEEVQKMNSKSNAPRKD----------DSSENEKGHLPMPNKSDN-

Figure 10
Vectors used for cloning

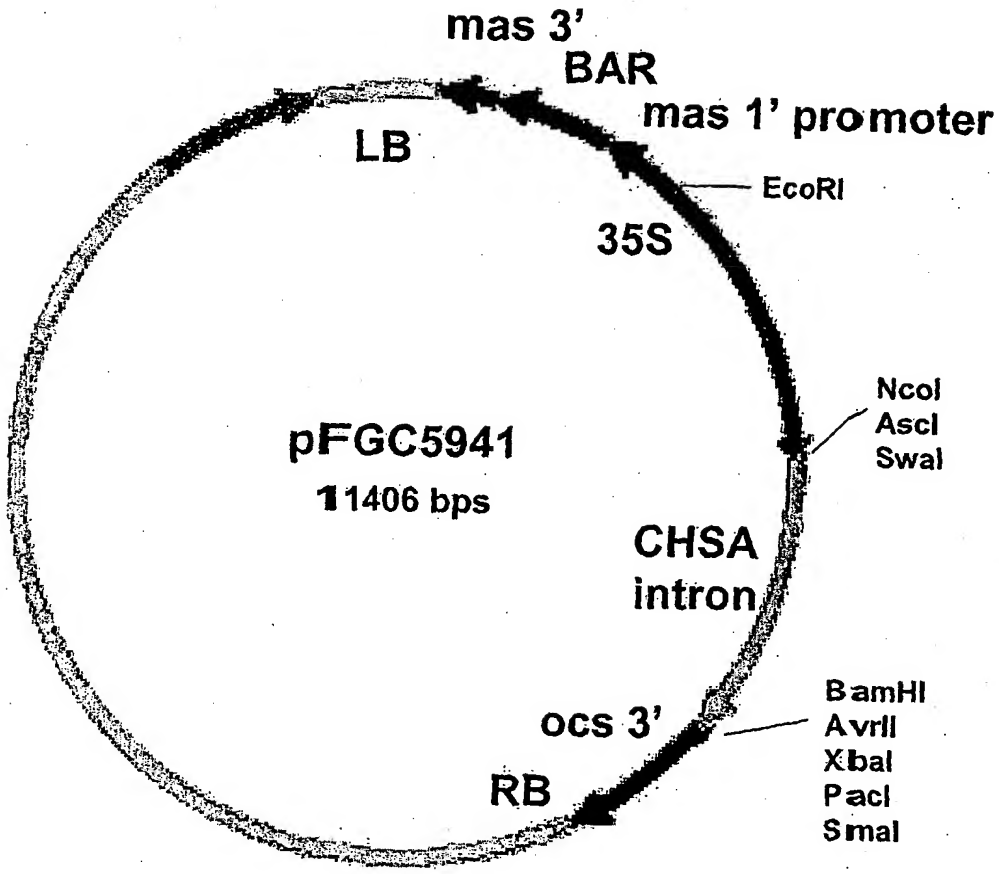
10A BJ60



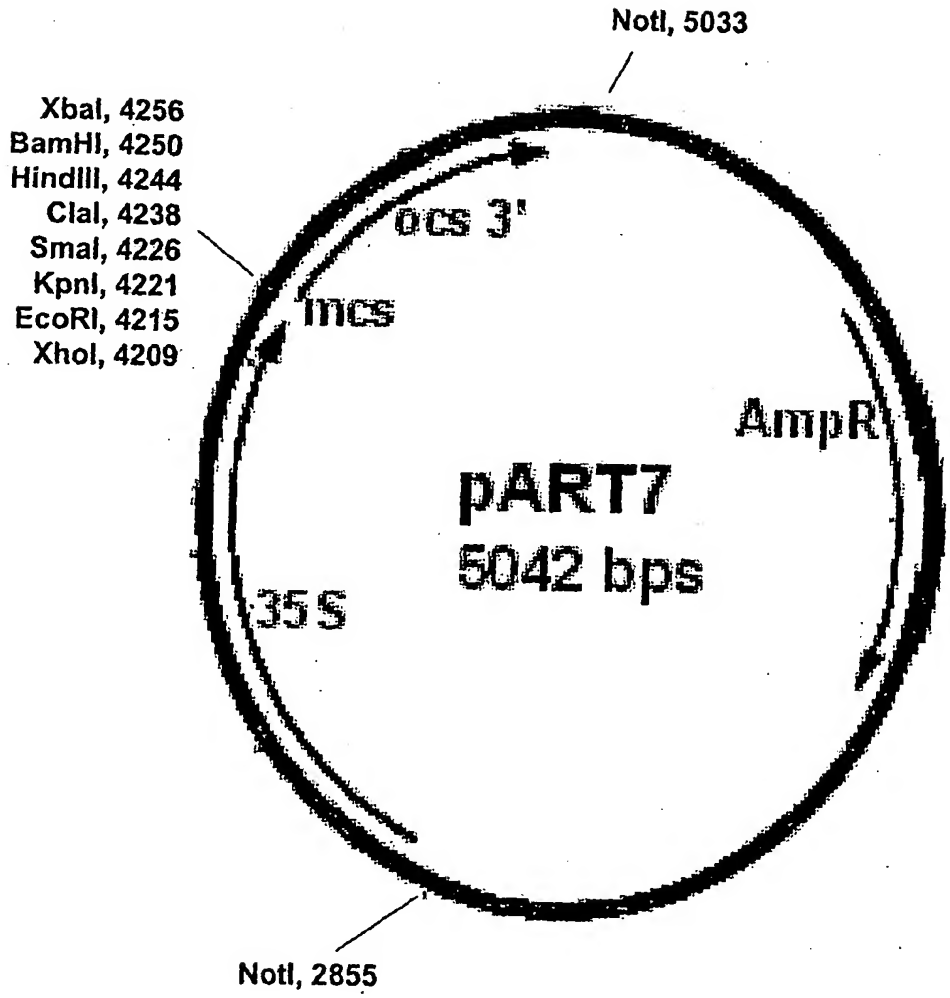
10B BJ40


SacI, 943
PstI, 961
NotI, 967
SacII, 987
SacII, 12508
RB
lacZ
Strep^R/Spec^R
SacII, 2639
35S
PstI, 2858
BJ40
12611 bps
nptII
Kan^R
PstI, 3476
ocs 3'
BamHI, 3488
HindIII, 3506
ClaI, 3512
LB
PstI, 5102

10C pFGC5941



10D pART7


NotI, 5033
XbaI, 4256
BamHI, 4250
HindIII, 4244
ClaI, 4238
SmaI, 4226
KpnI, 4221
EcoRI, 4215
XhoI, 4209
ocs 3'
mcs
AmpR
pART7
5042 bps
35 S
NotI, 2855

0E BJ36

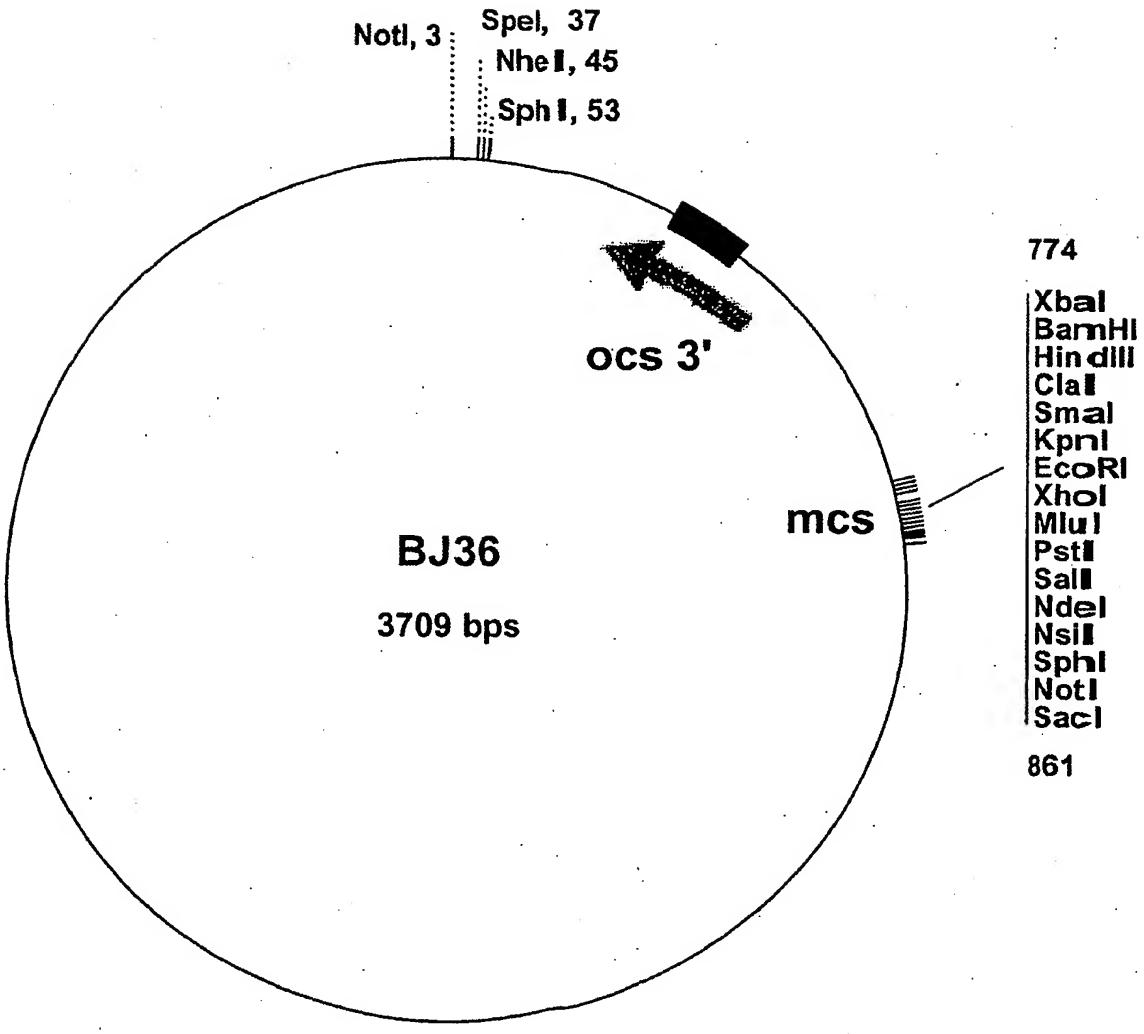
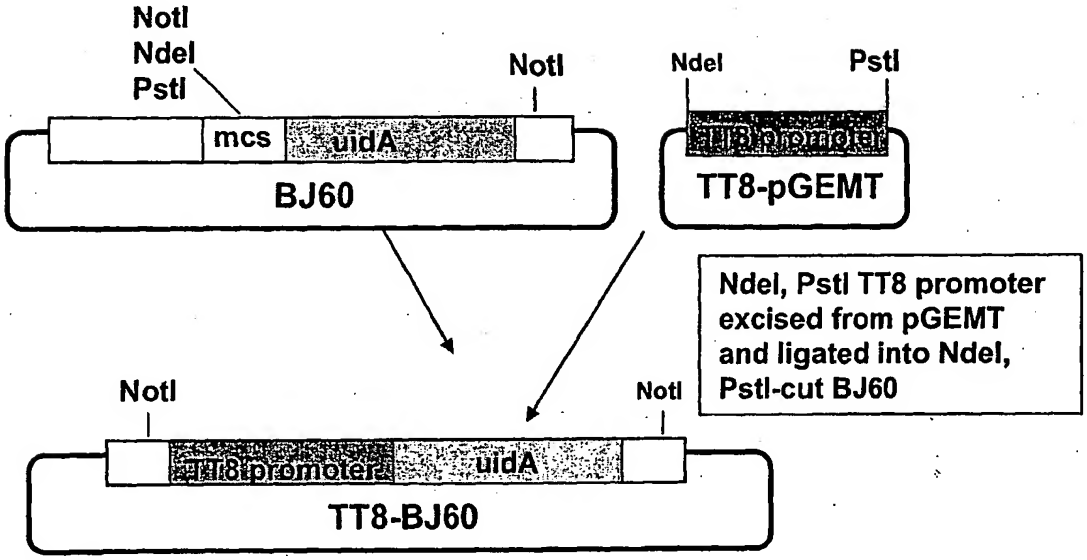

NotI, 3
SpeI, 37
NheI, 45
SphI, 53
ocs 3'
mcs
BJ36
3709 bps
774
XbaI
BamHI
HindIII
ClaI
SmaI
KpnI
EcoRI
XhoI
MluI
PstI
SalI
NdeI
NsiI
SphI
NotI
SacI
861

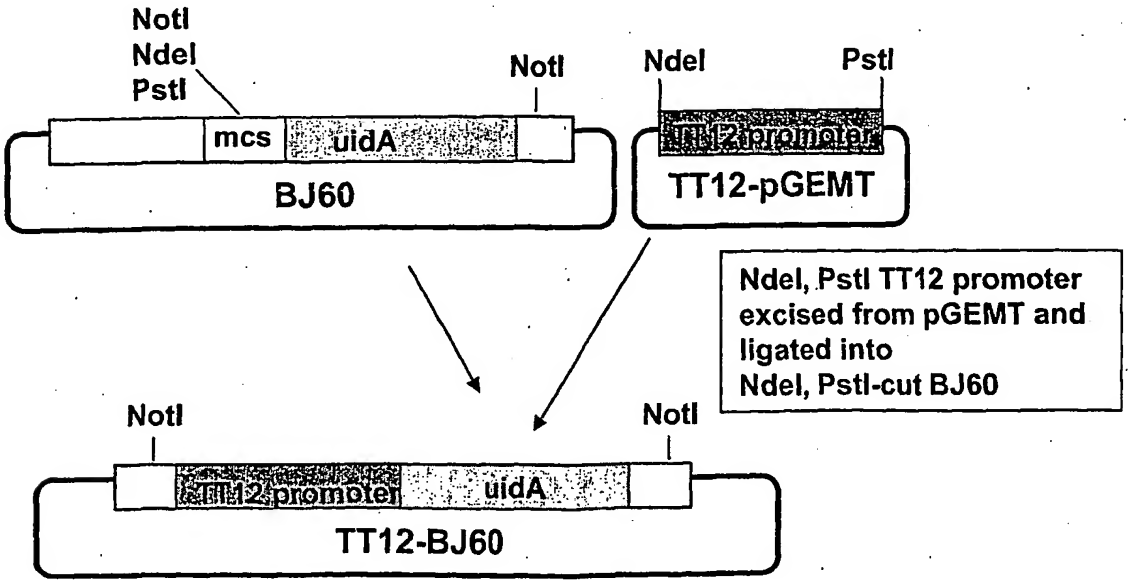
Figure 11

Cloning strategy, Example 3

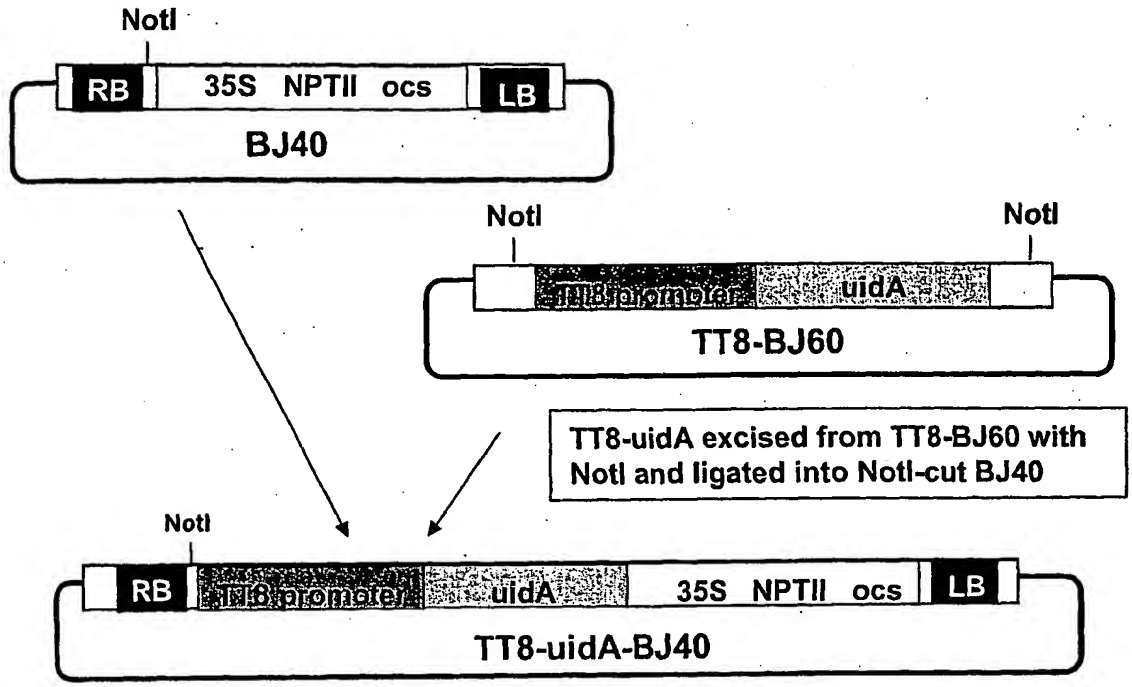
Example 3a(i)



Example 3a(ii)



Example 3b(i)



Example 3b(ii)

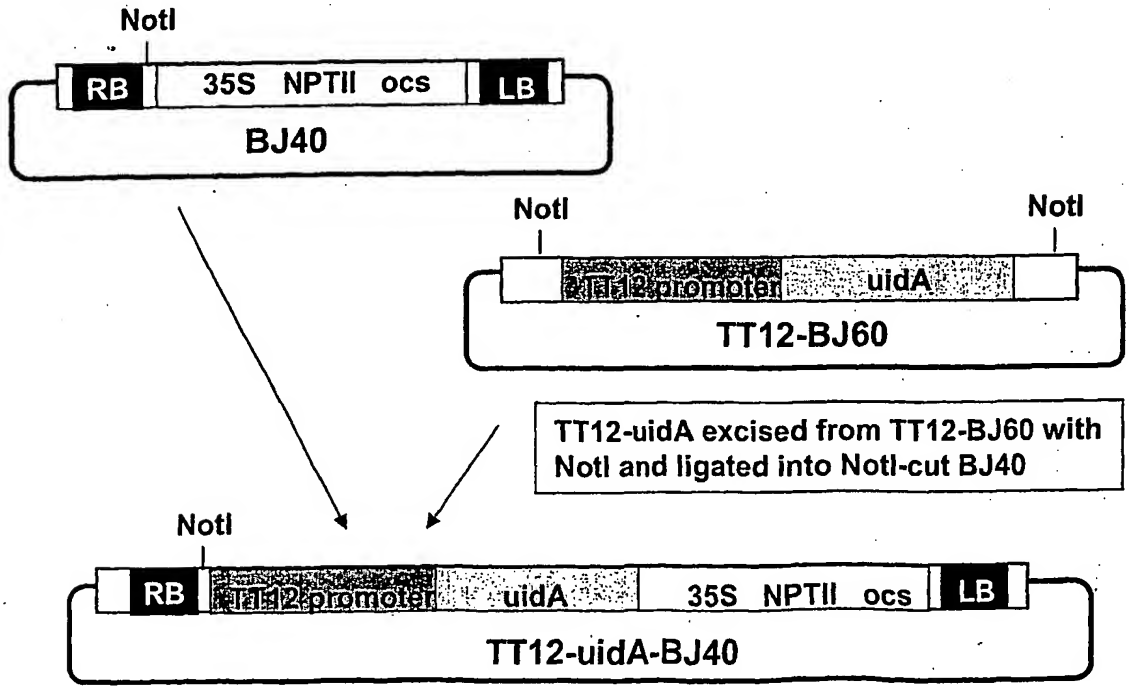


Figure12

TT12::uidA

Figure 13A

Cloning strategy, Example 4

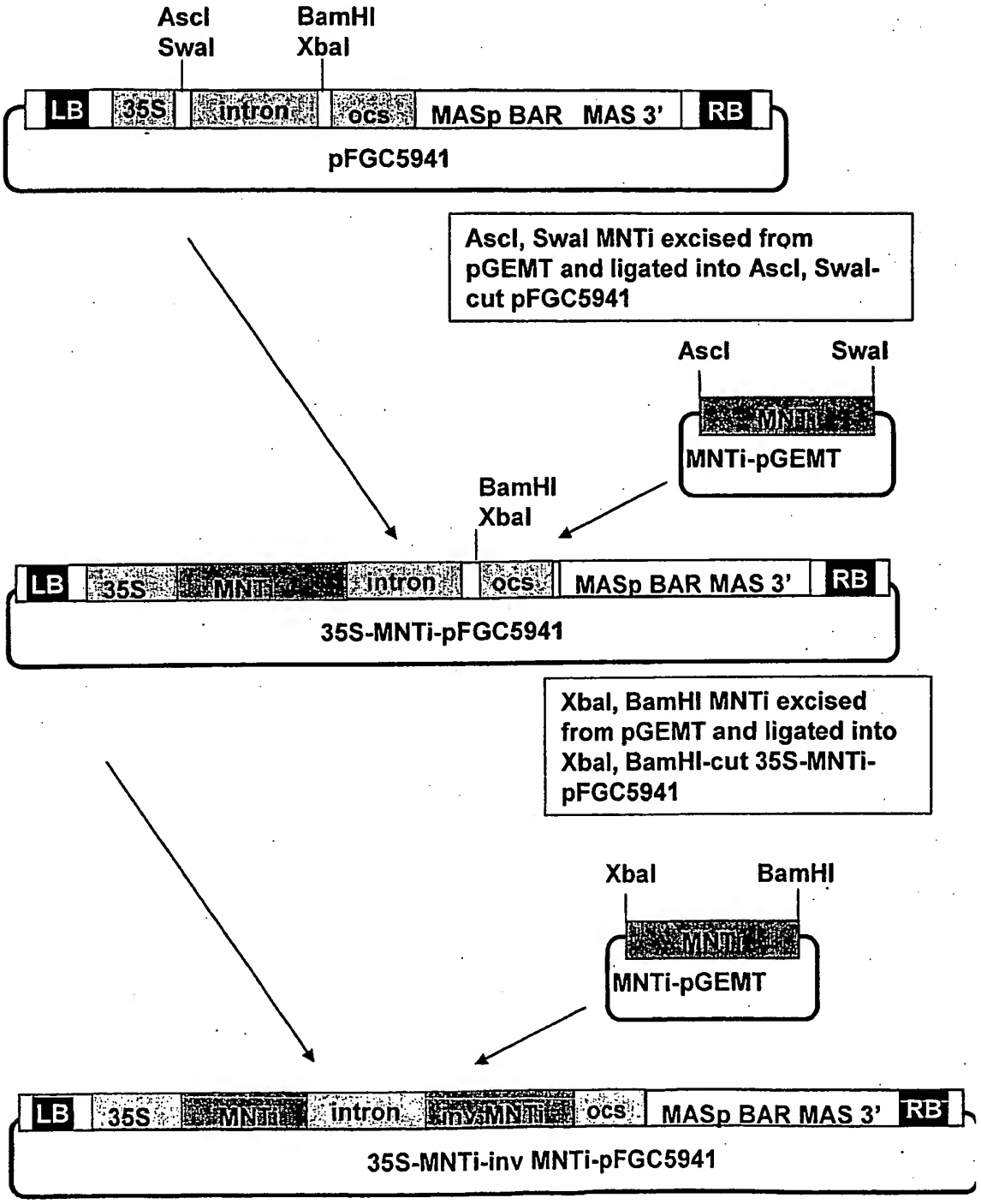


Figure 13B

Plants transformed with the *35S::MNT* RNAi vector Example 4

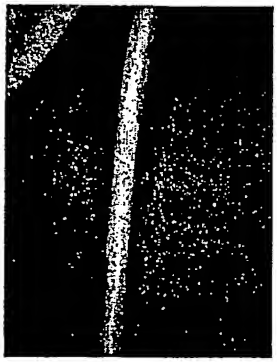
Primary inflorescence

wild-type Col-3

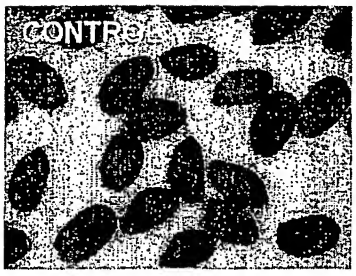
35S::MNT RNAi line 3

Primary inflorescence stem

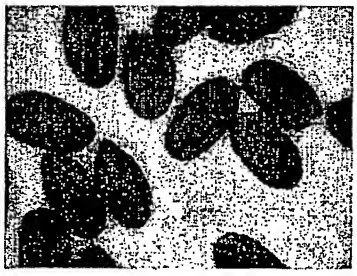
wild-type Col-3



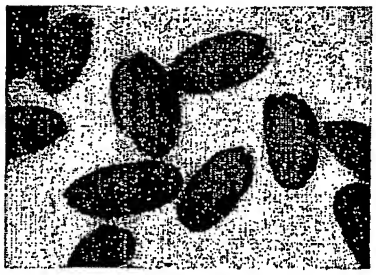
35S::MNT RNAi line 3



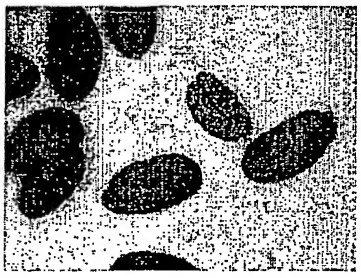
wild-type Col-3
mean wt 13.8 µg



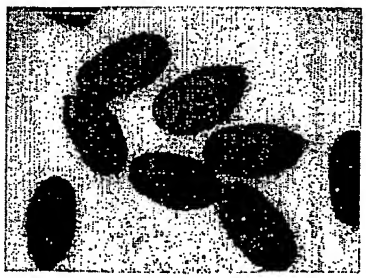
35S::MNT RNAi line 1
mean wt 34.0 µg



35S::MNT RNAi line 2
mean wt 35.6 µg



35S::MNT RNAi line 3
mean wt 34.8 µg



35S::MNT RNAi line 4
mean wt 36.7 µg

Figure 14

Cloning strategy, Example 5

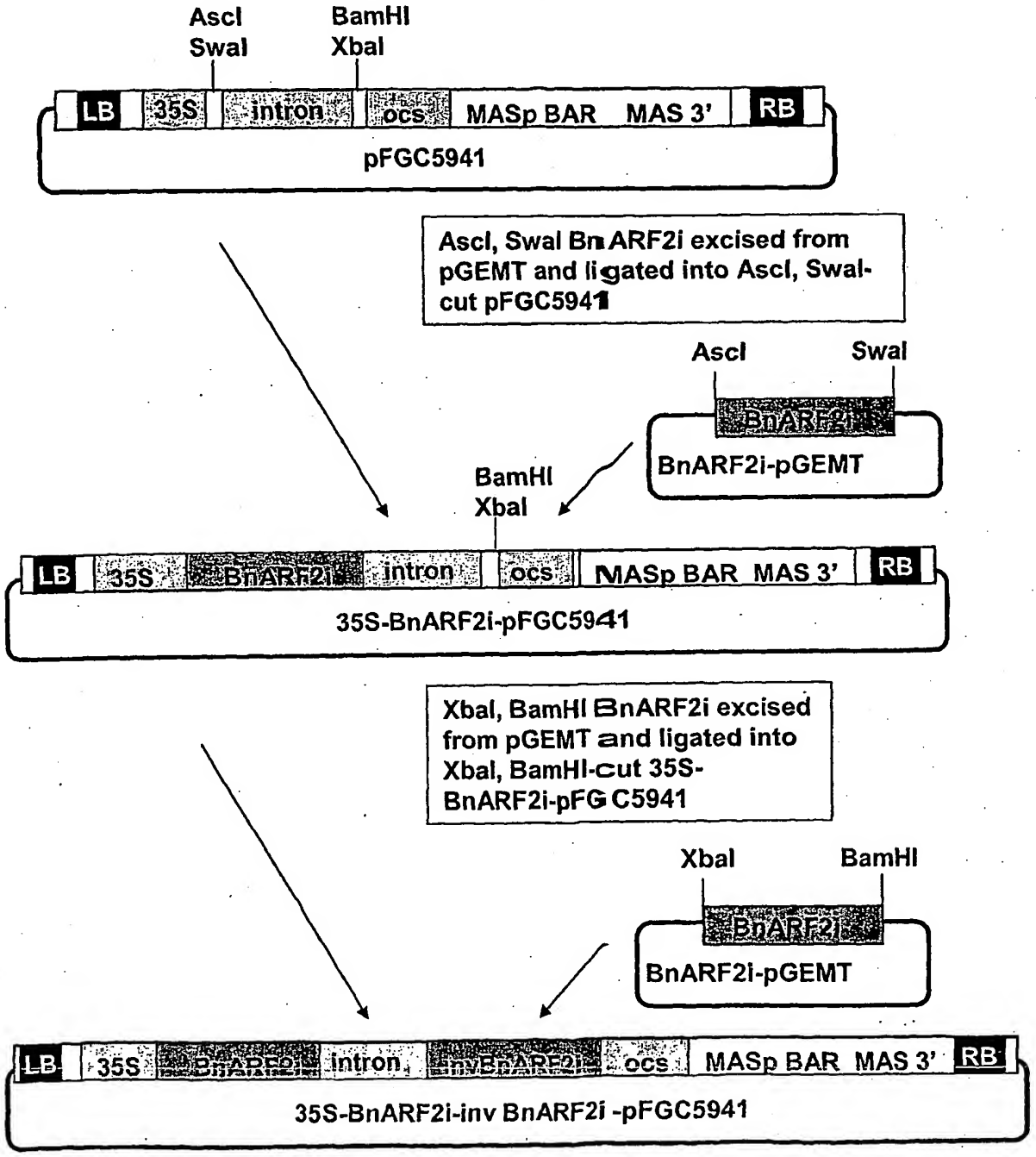
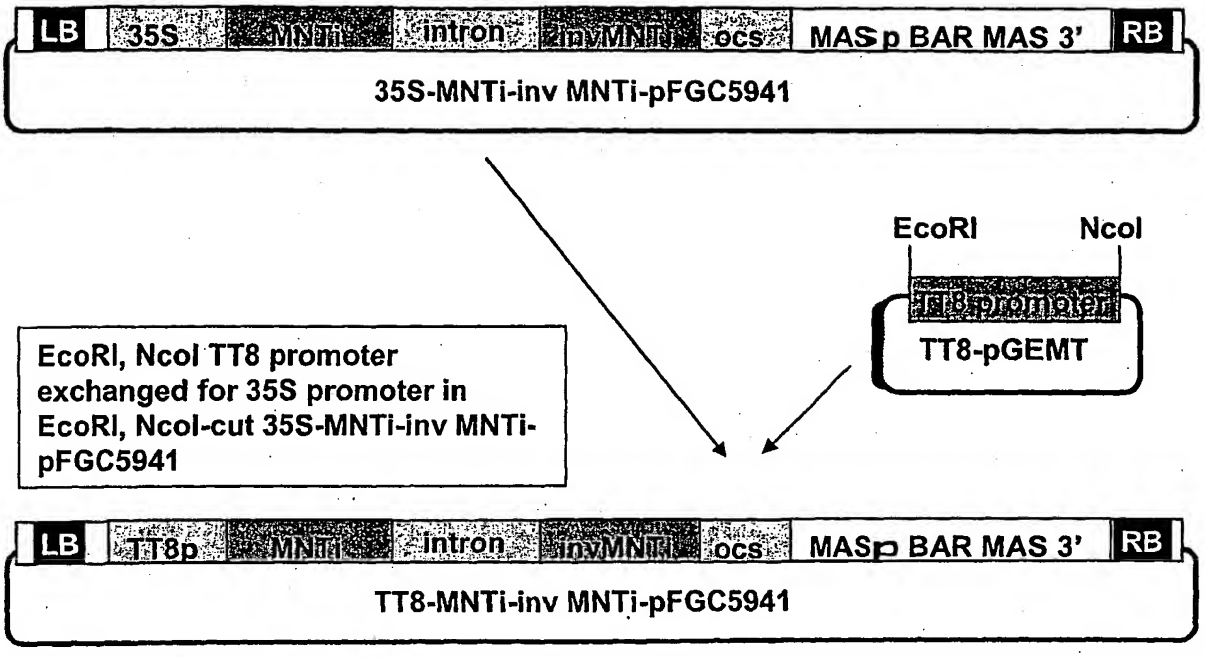


Figure 15
Cloning strategy, Example 6

Example 6a(i)



Example 6a(ii)

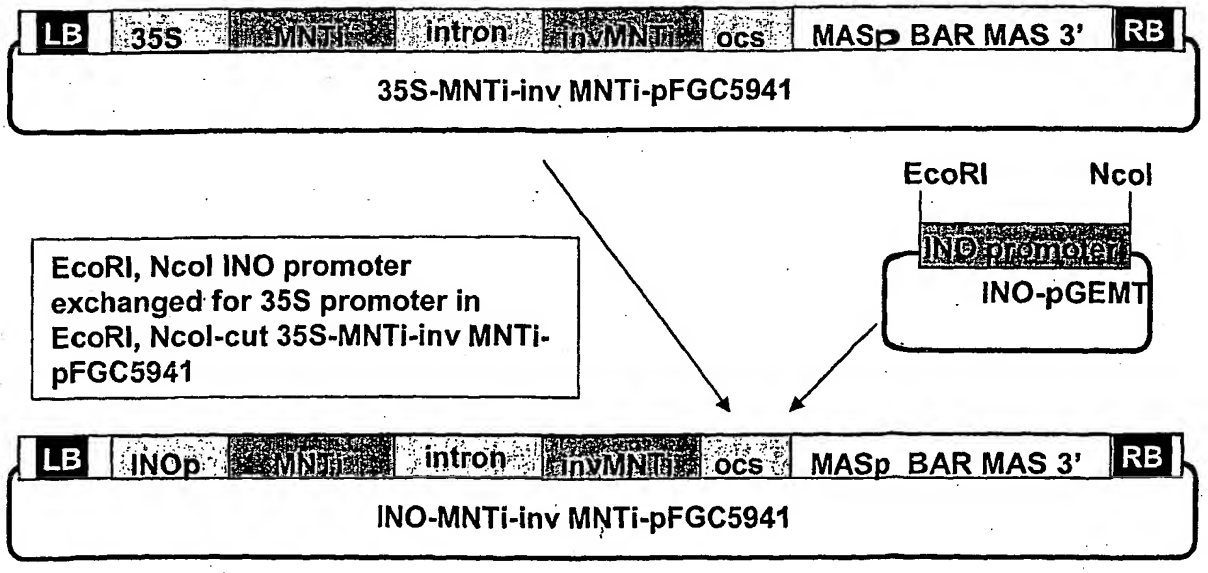
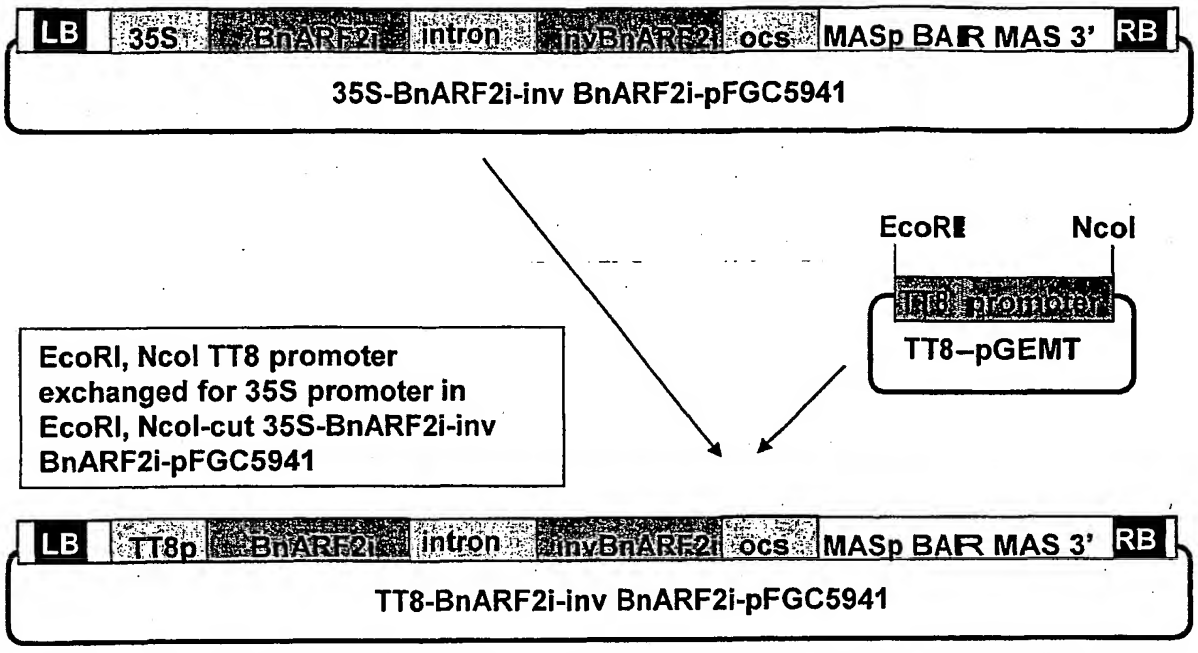


Figure 16
Cloning strategy, Example 7

Example 7a(i)



Example 7a(ii)

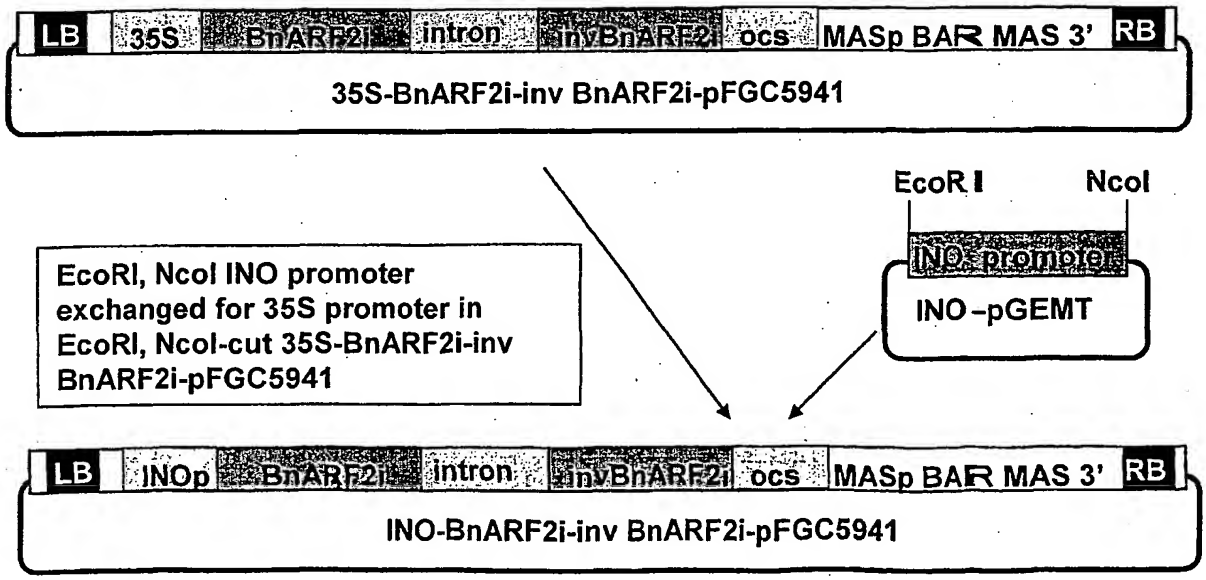
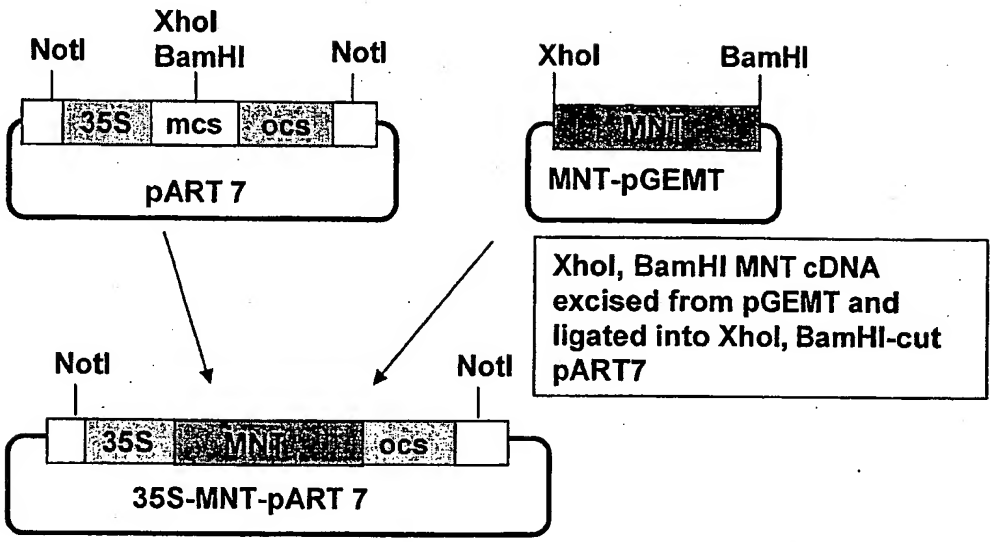
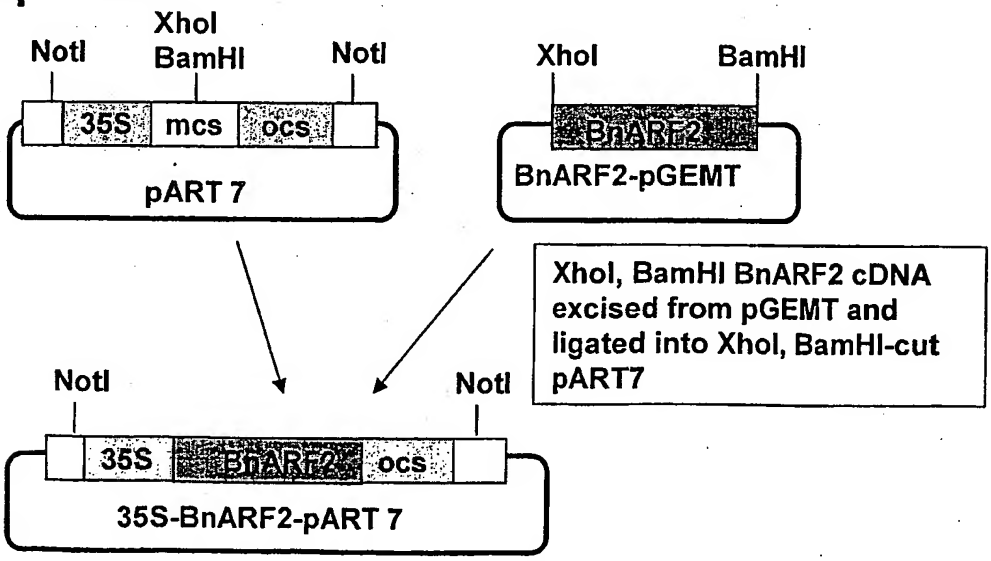


Figure 17A
Cloning strategy, Examples 8, 9

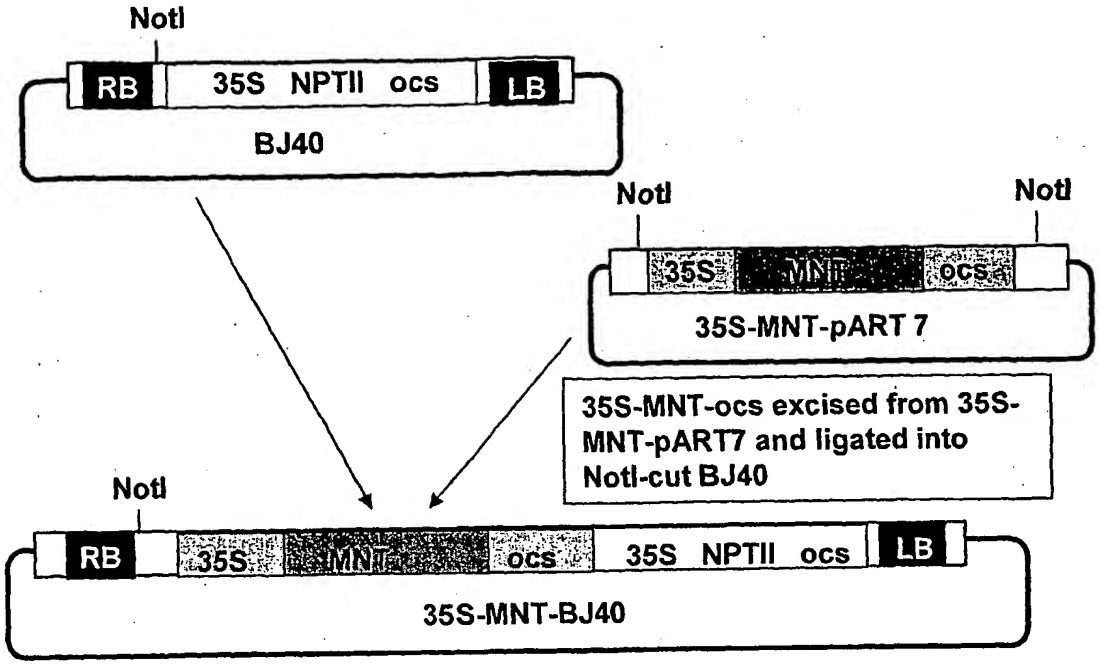
Example 8a



Example 9a



Example 8b



Example 9b

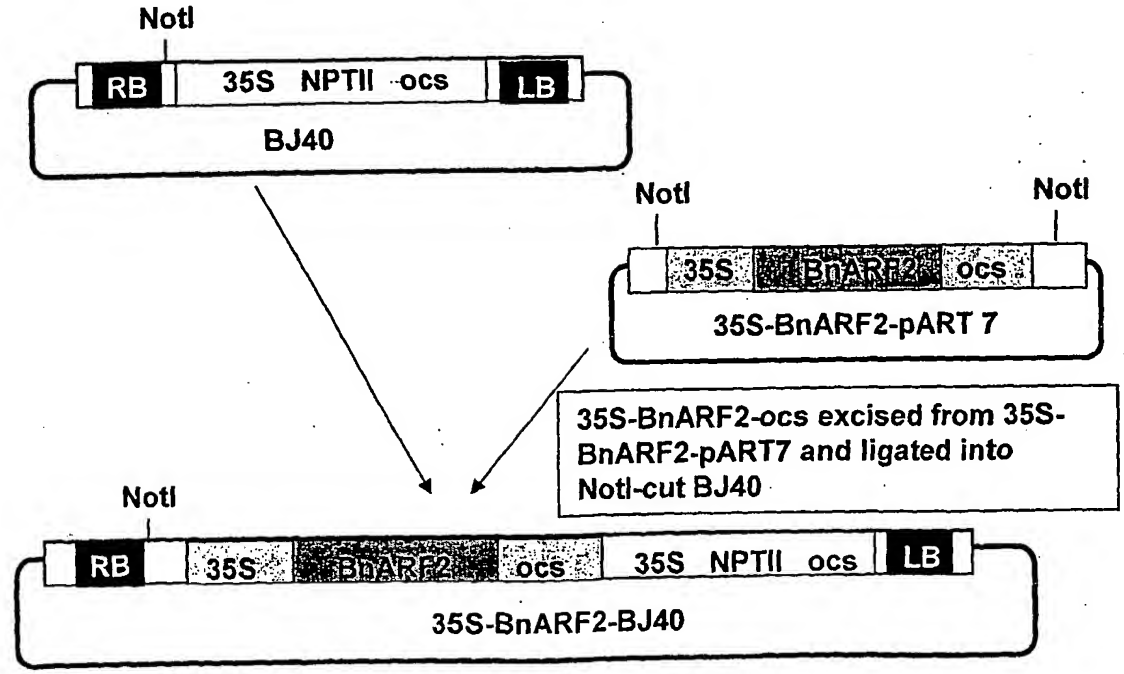
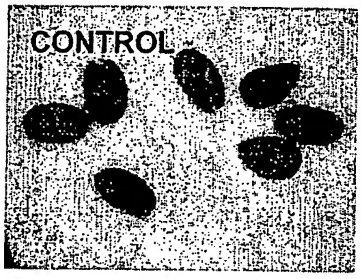


Figure 17B

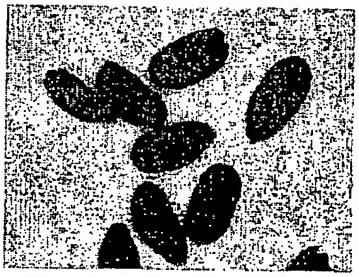
Analysis of wild-type plants transformed with the *35S::MNT* vector

Example 8

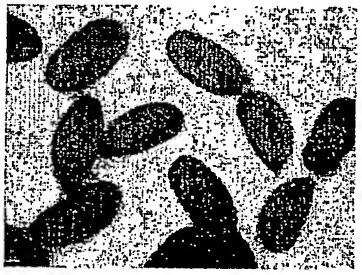
35S::MNT



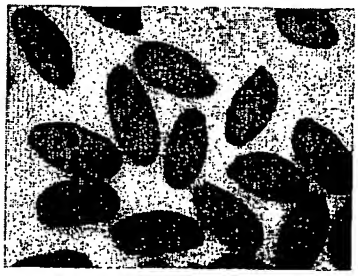
wild-type Col-3
mean wt 15.0 µg



35S::MNT line 1
mean wt 23.1 µg



35S::MNT line 2
mean wt 28.7 µg



35S::MNT line 3
mean wt 24.6 µg

Semiquantitative RT-PCR

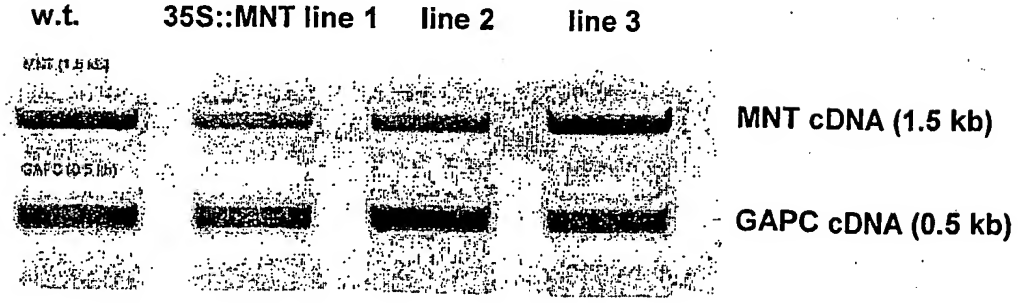
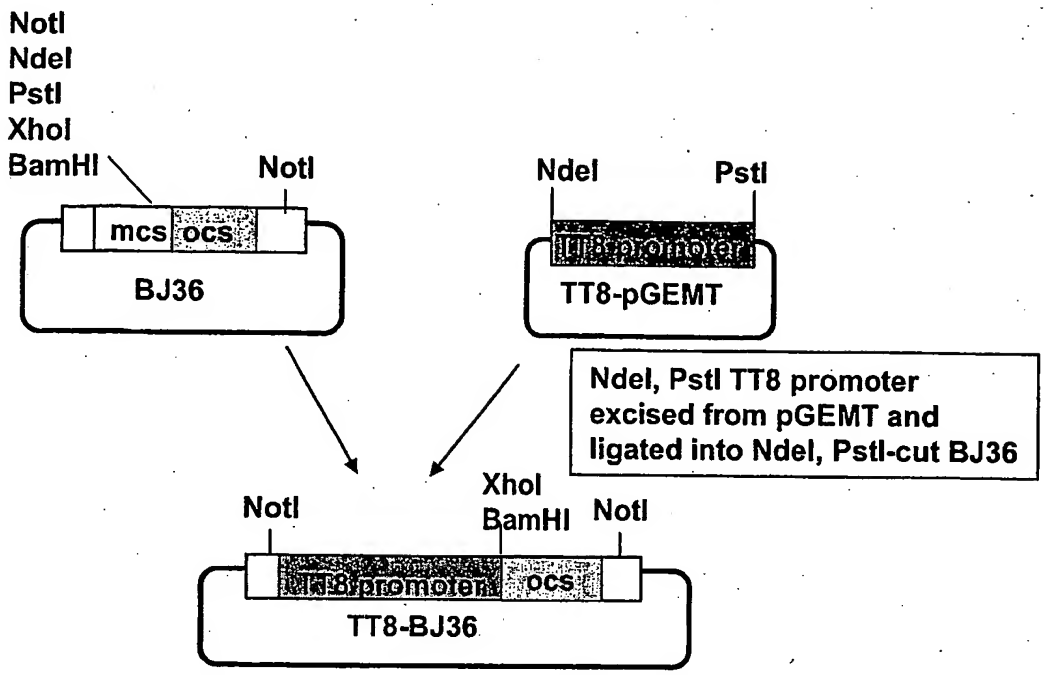


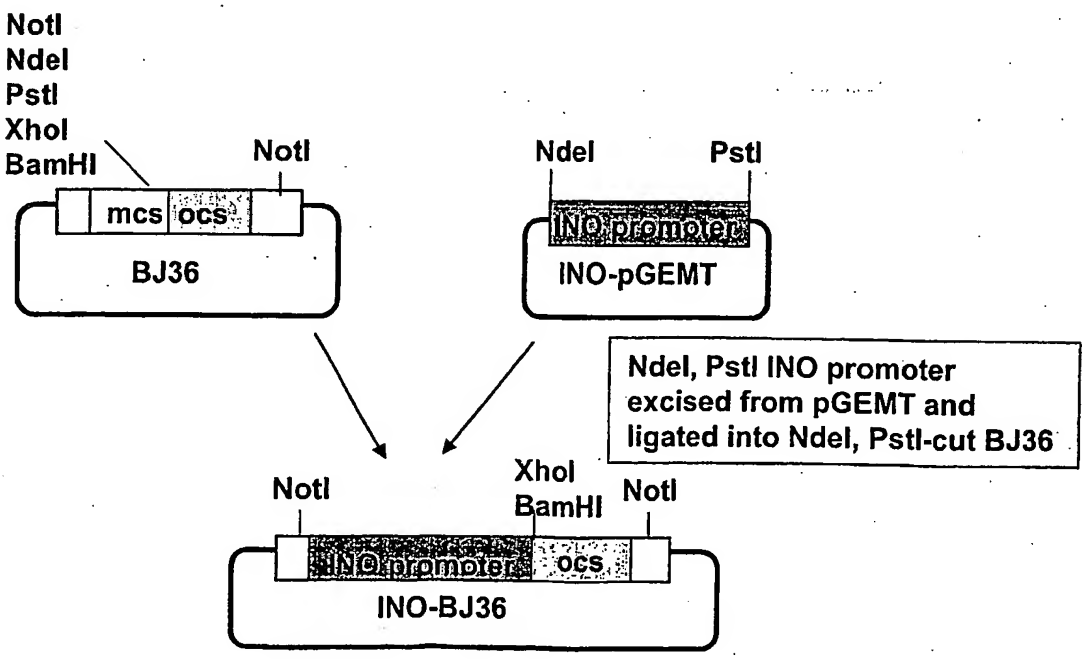
Figure 18

Cloning strategy, Example 10

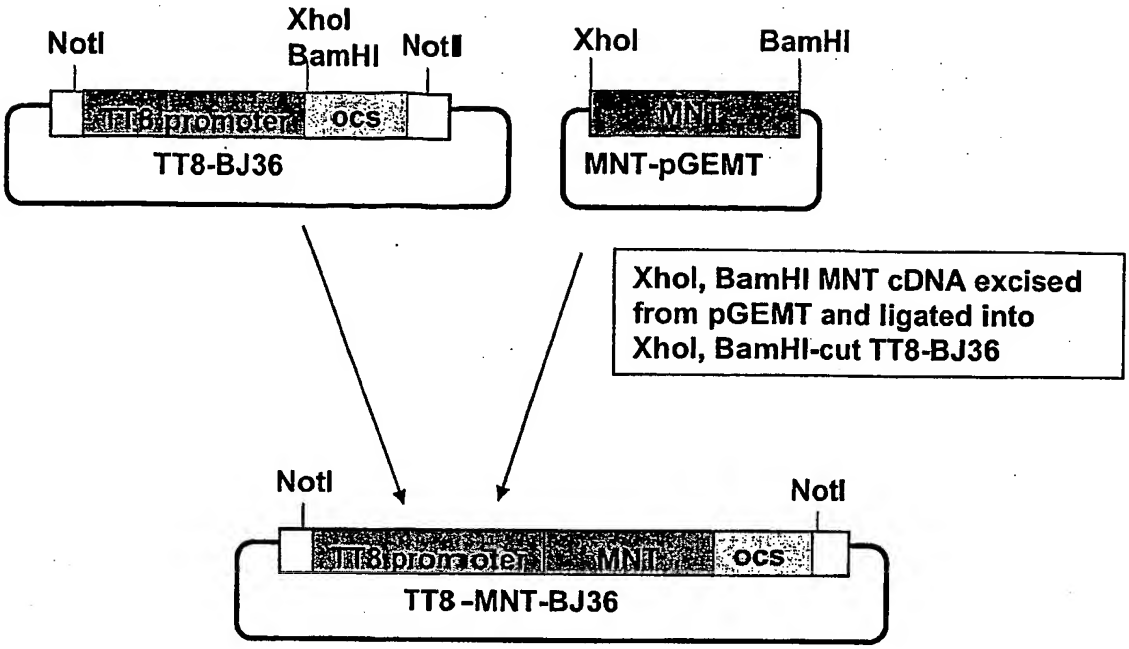
Example 10a(i)



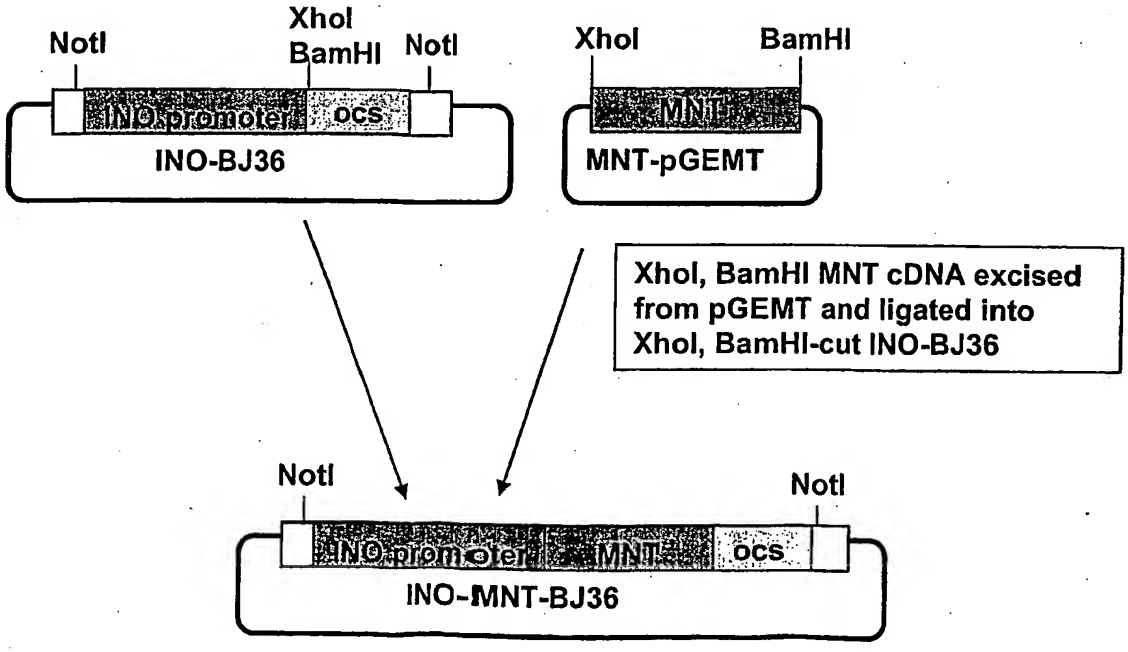
Example 10a(ii)



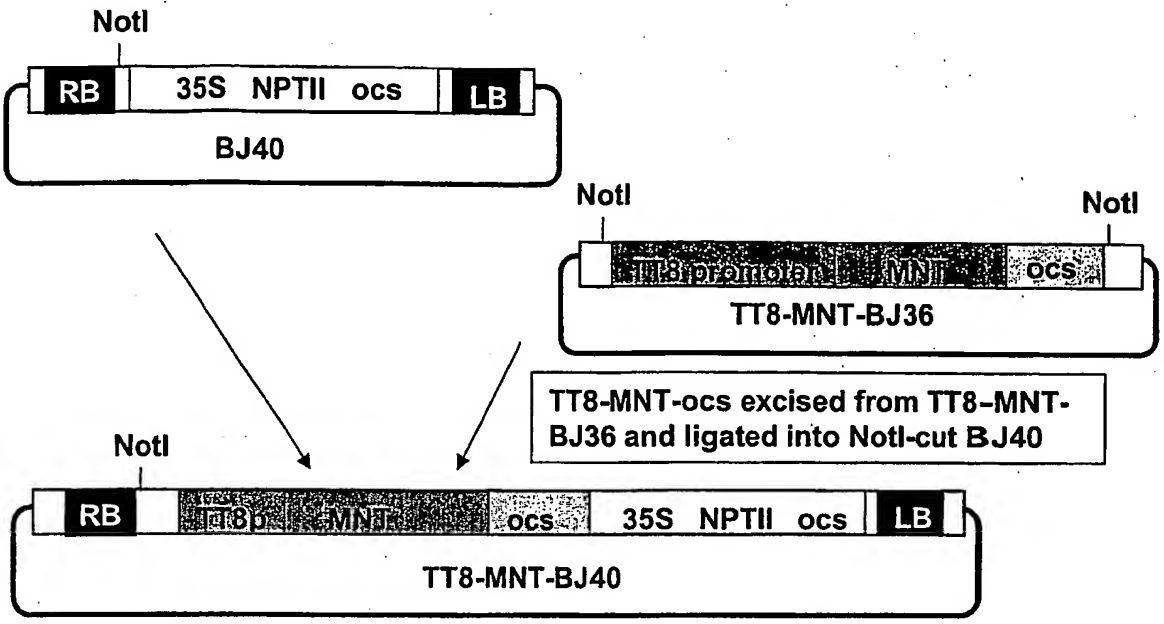
Example 10b(i)



Example 10b(ii)



Example 10c(i)



Example 10c(ii)

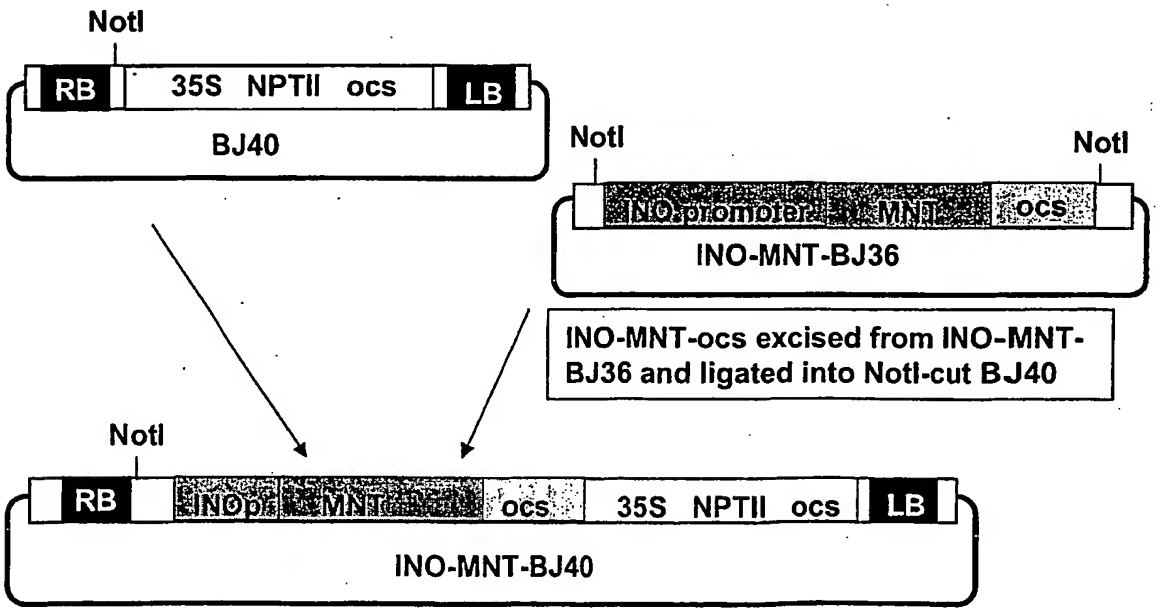
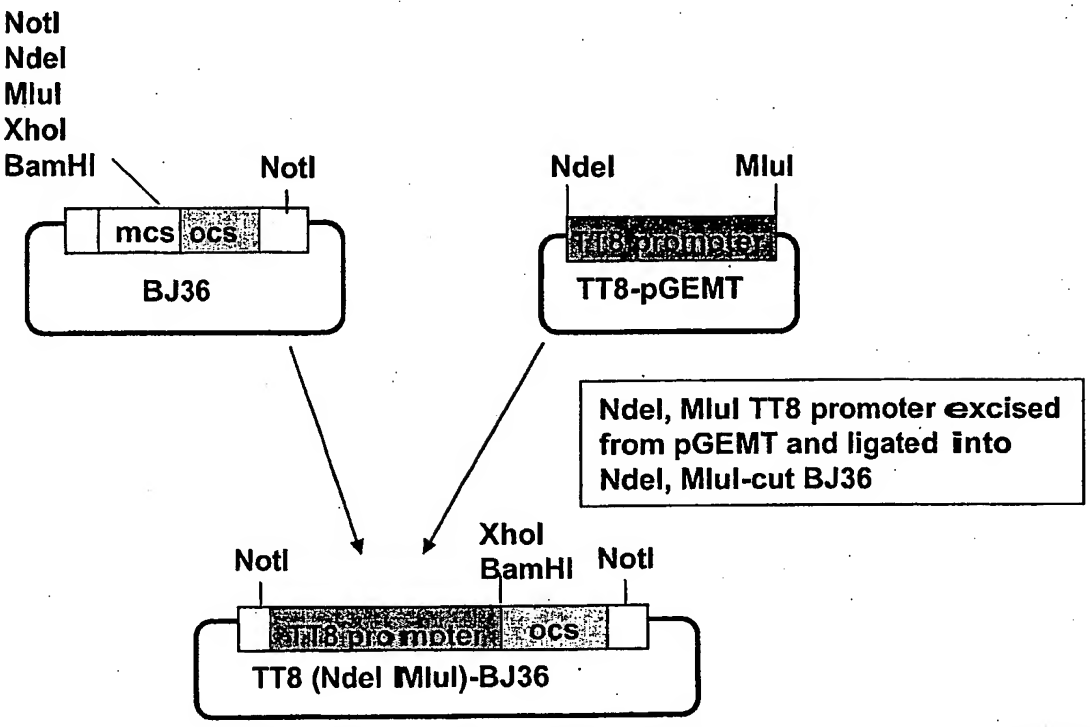


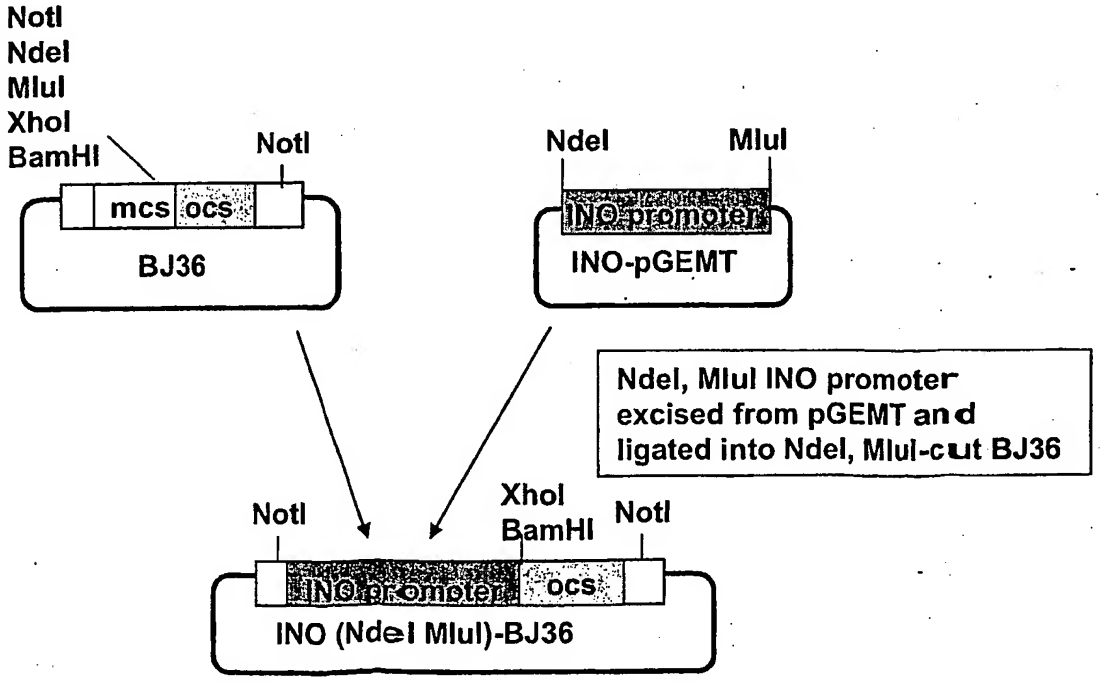
Figure 19

Cloning strategy, Example 11

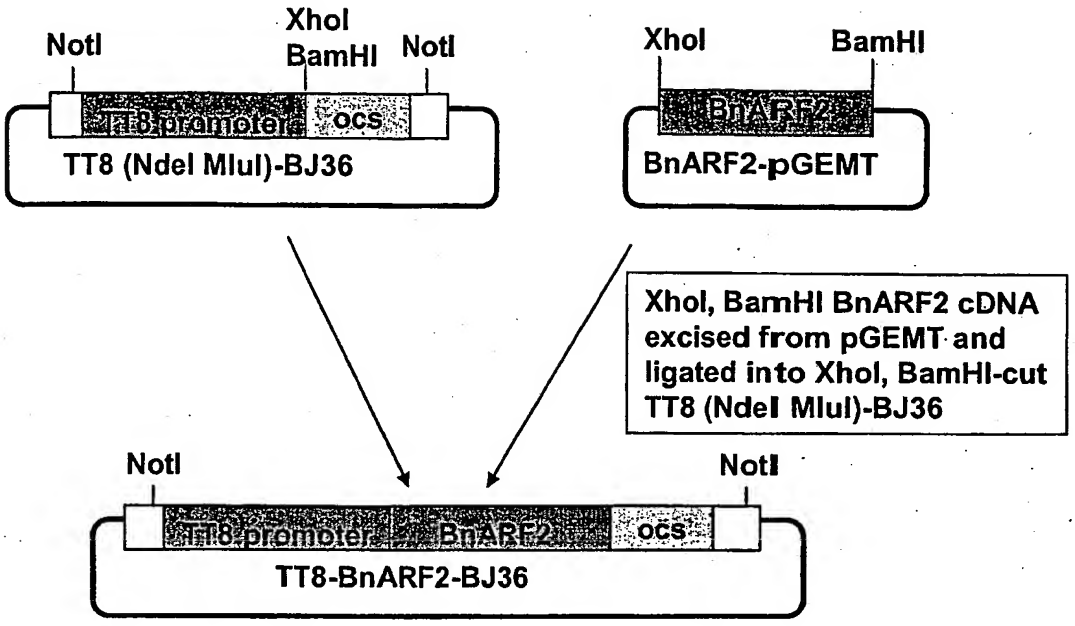
Example 11a(i)



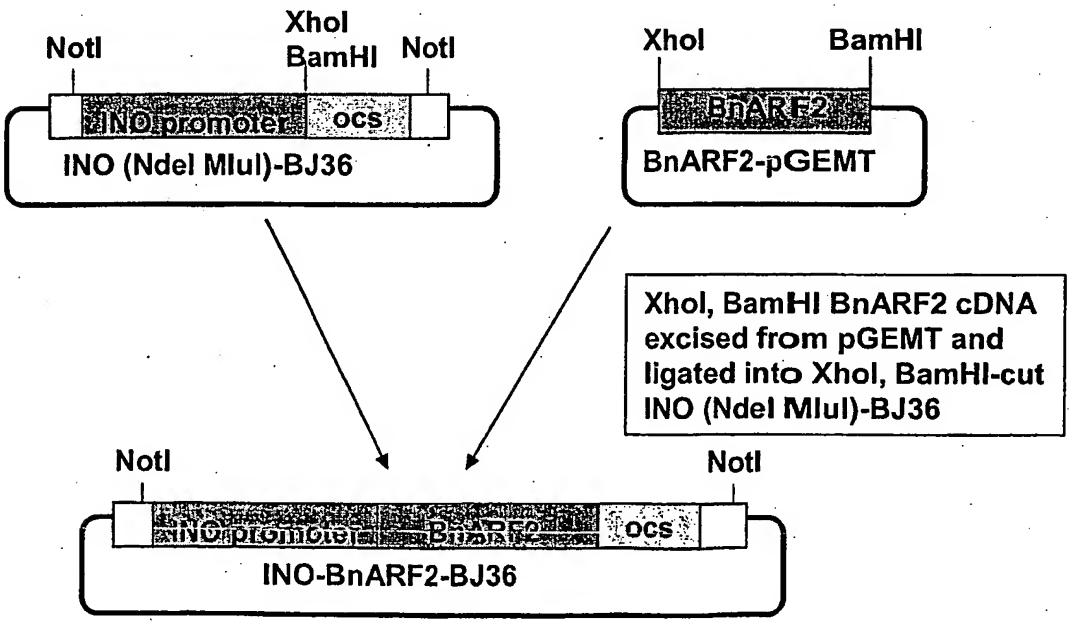
Example 11a(ii)



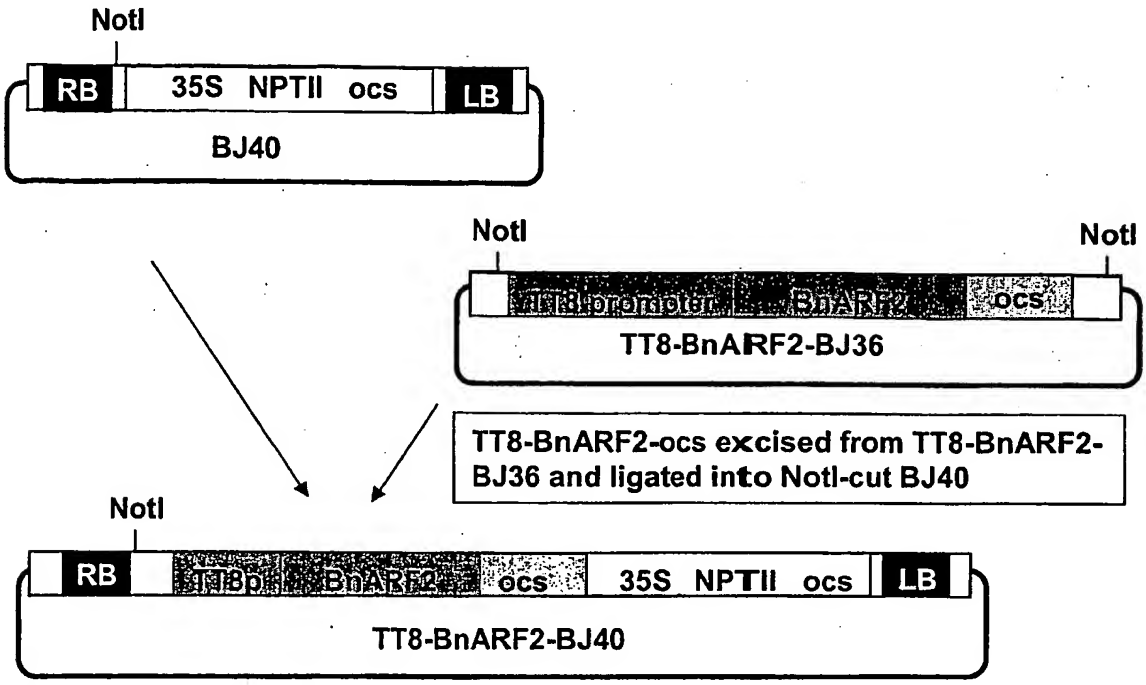
Example 11b(i)



Example 11b(ii)



Example 11c(i)



Example 11c(ii)

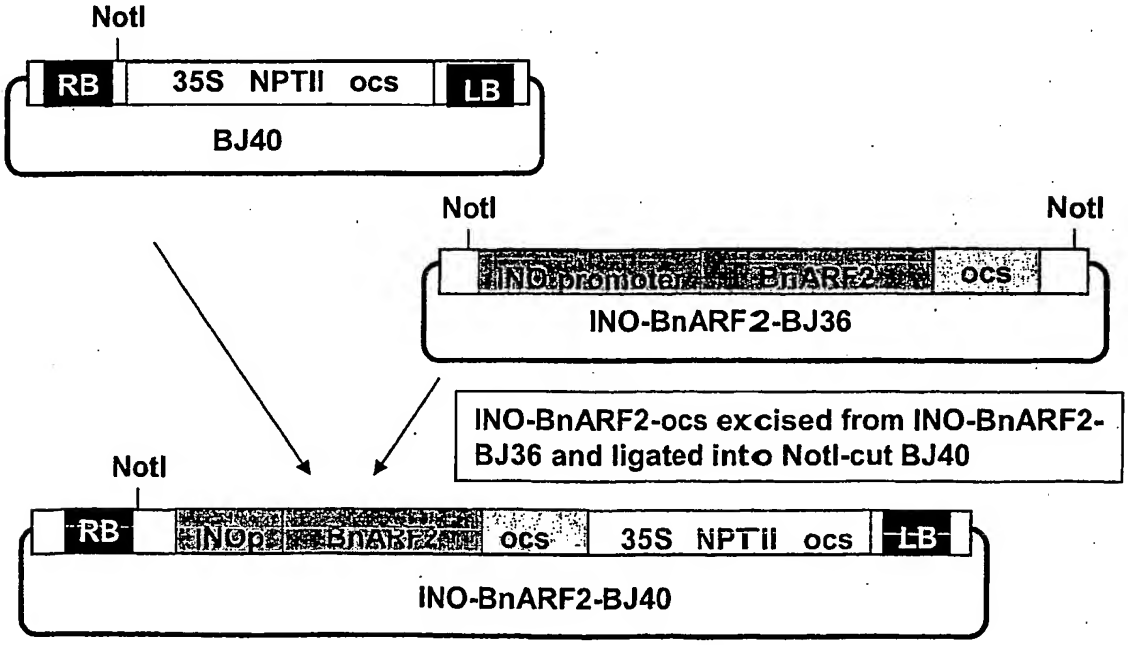
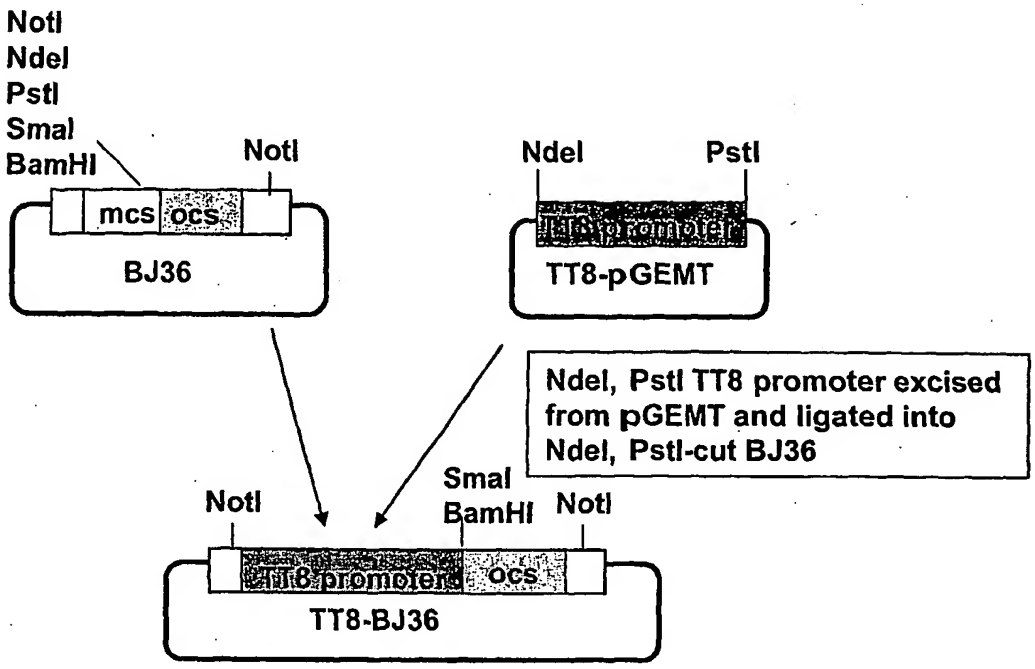


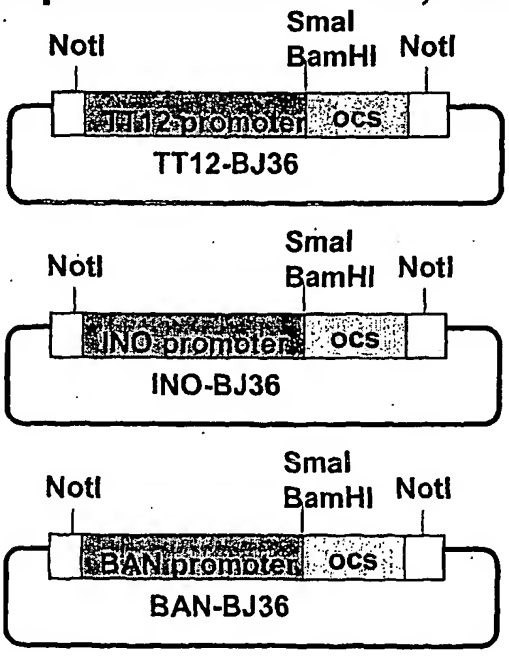
Figure 20

Cloning strategy, Examples 12, 13

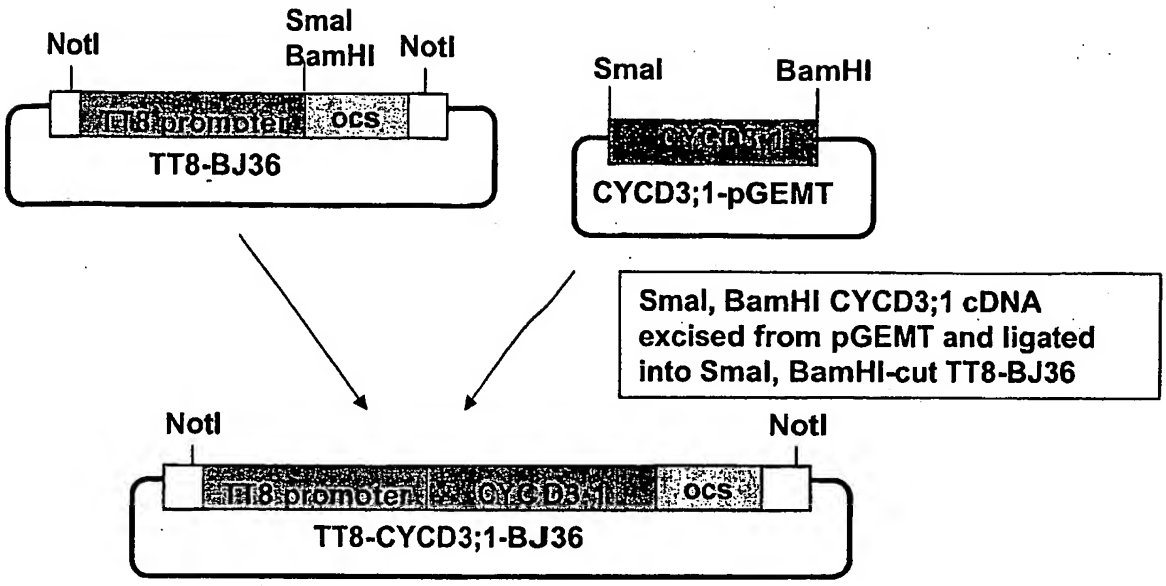
Examples 12a, 13a



Repeat process with TT12, INO, BAN promoters



Examples 12b, 13b



Repeat process with IPT1, ANT, CYCB1;1 cDNAs and TT12, INO, BAN promoters

TT8-IPT1-BJ40

TT8-ANT-BJ40

TT8-CYCB1;1-BJ40

TT12-CYCD3;1-BJ40

TT12-IPT1-BJ40

TT12-ANT-BJ40

TT12-CYCB1;1-BJ40

INO-CYCD3;1-BJ40

INO-IPT1-BJ40

INO-ANT-BJ40

INO-CYCB1;1-BJ40

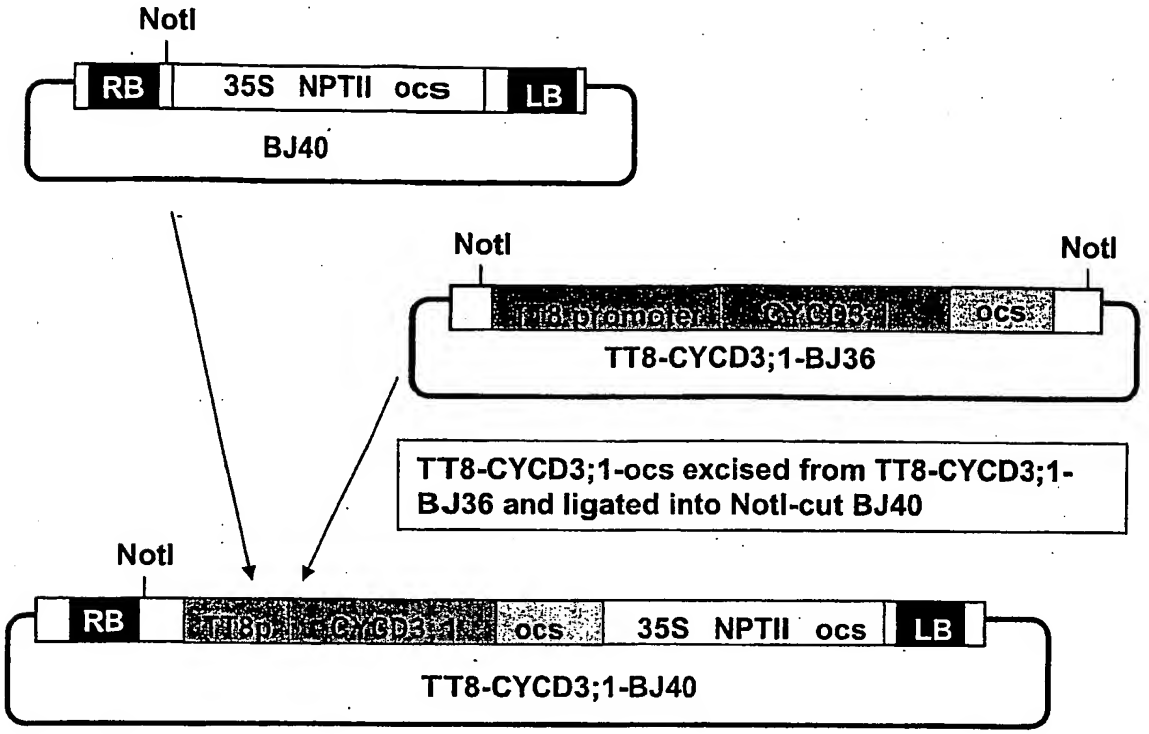
BAN-CYCD3;1-BJ40

BAN-IPT1-BJ40

BAN-ANT-BJ40

BAN-CYCB1;1-BJ40

Example 12c, 13c



Repeat process with all BJ36 constructs shown in Example 12b

TT8-IPT1-BJ40

TT8-ANT-BJ40

TT8-CYCB1;1-BJ40

TT12-CYCD3;1-BJ40

TT12-IPT1-BJ40

TT12-ANT-BJ40

TT12-CYCB1;1-BJ40

INO-CYCD3;1-BJ40

INO-IPT1-BJ40

INO-ANT-BJ40

INO-CYCB1;1-BJ40

BAN-CYCD3;1-BJ40

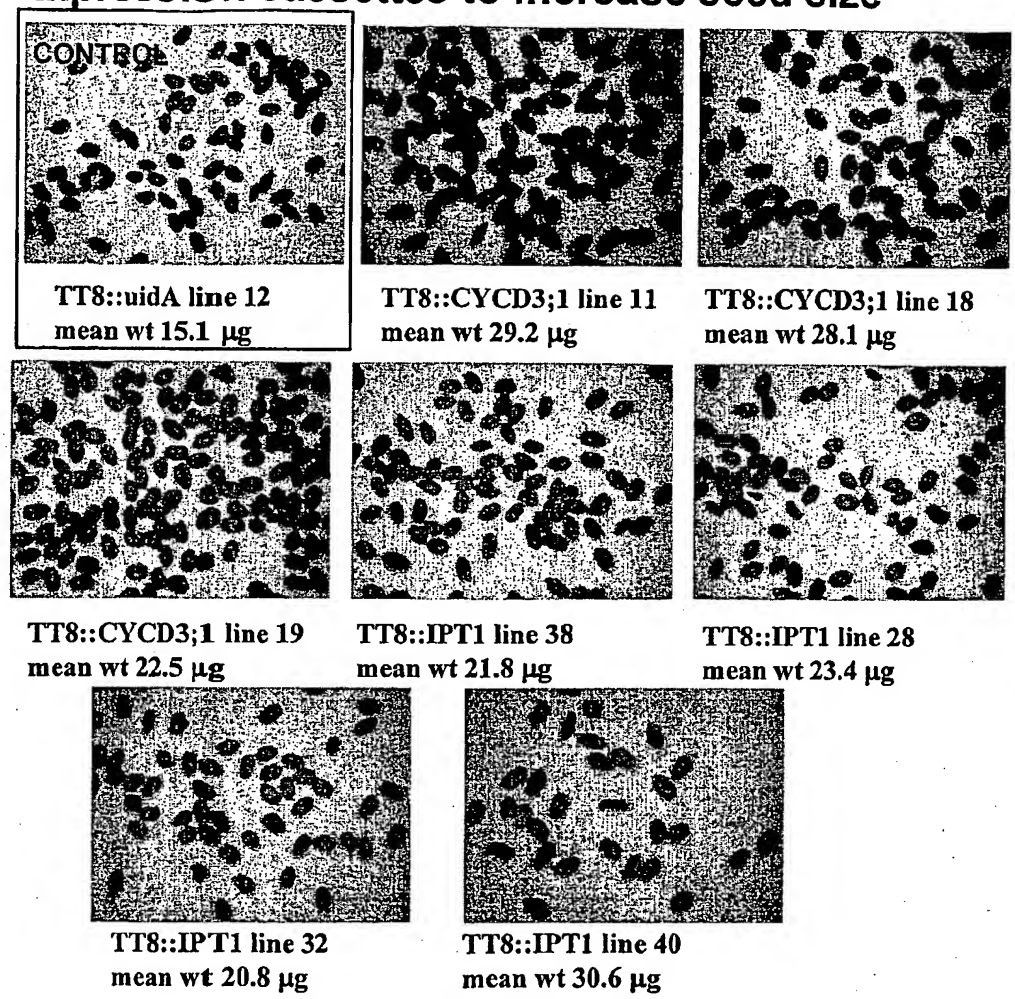
BAN-IPT1-BJ40

BAN-ANT-BJ40

BAN-CYCB1;1-BJ40

Figure 21A

Expression cassettes to increase seed size



Distribution of seed weights in TT8::uidA (control), TT8::CYCD3;1, and TT8::IPT1families

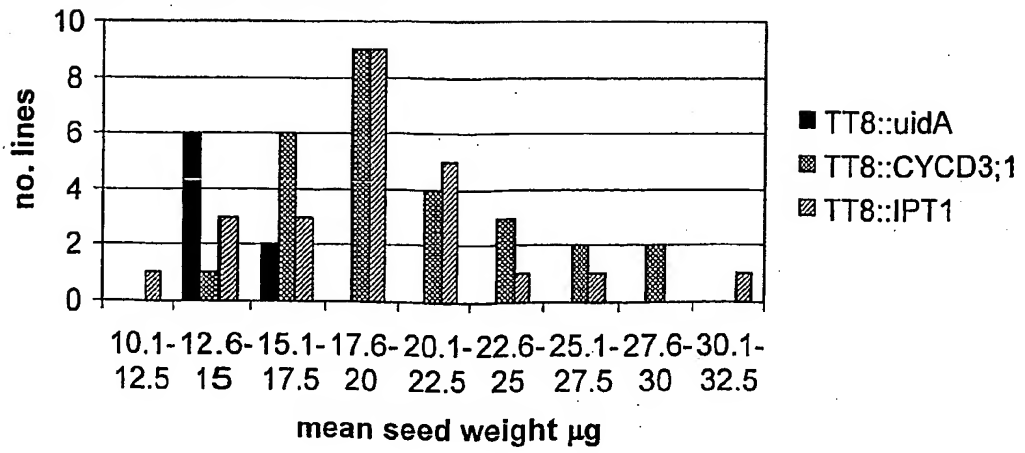
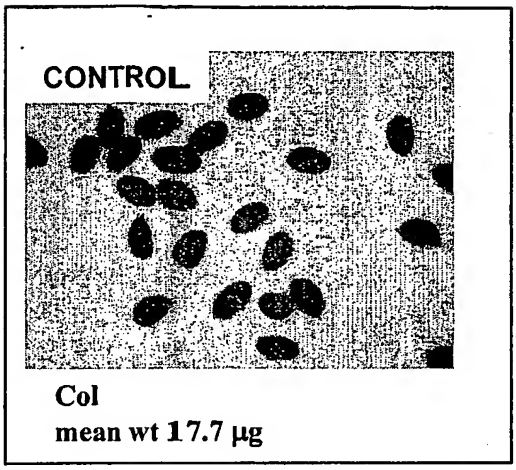
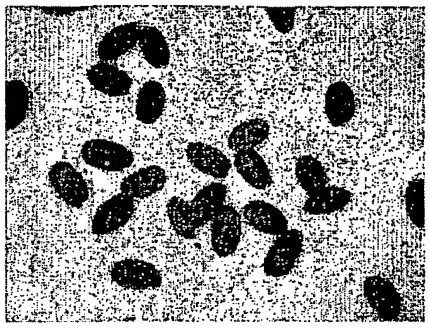


Figure 21B

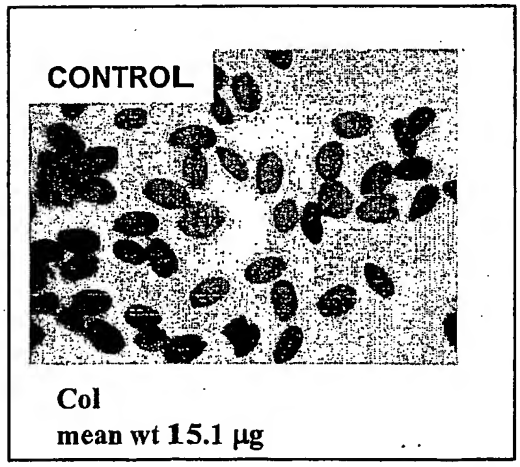
Expression cassettes to increase seed size



Col
mean wt 17.7 µg



BAN::CYCD3;1 line 1
mean wt 23.9 µg



Col
mean wt 15.1 µg

INO::IPT1 line 9
mean wt 23.1 µg

Figure 22
Cloning strategy, Example 14

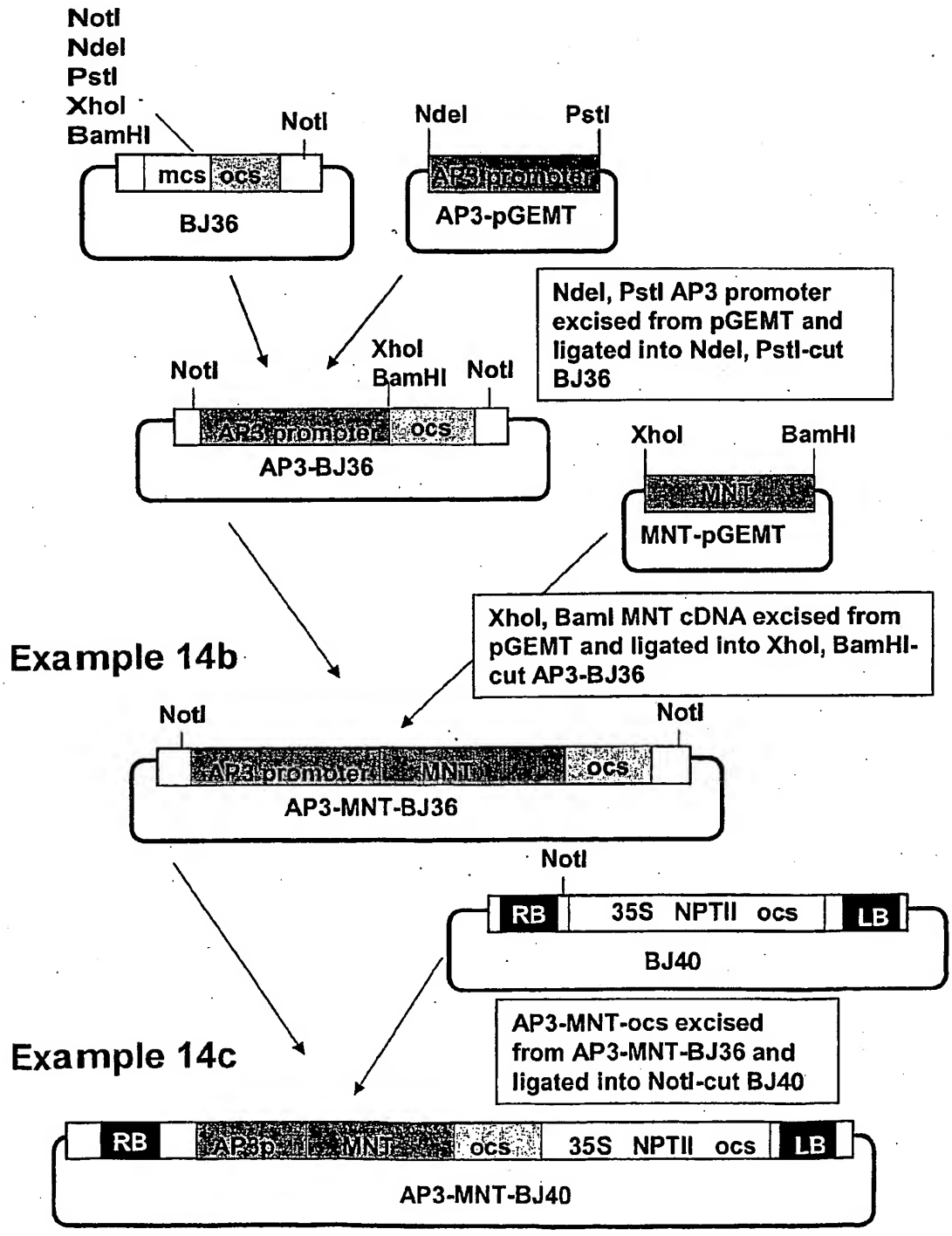
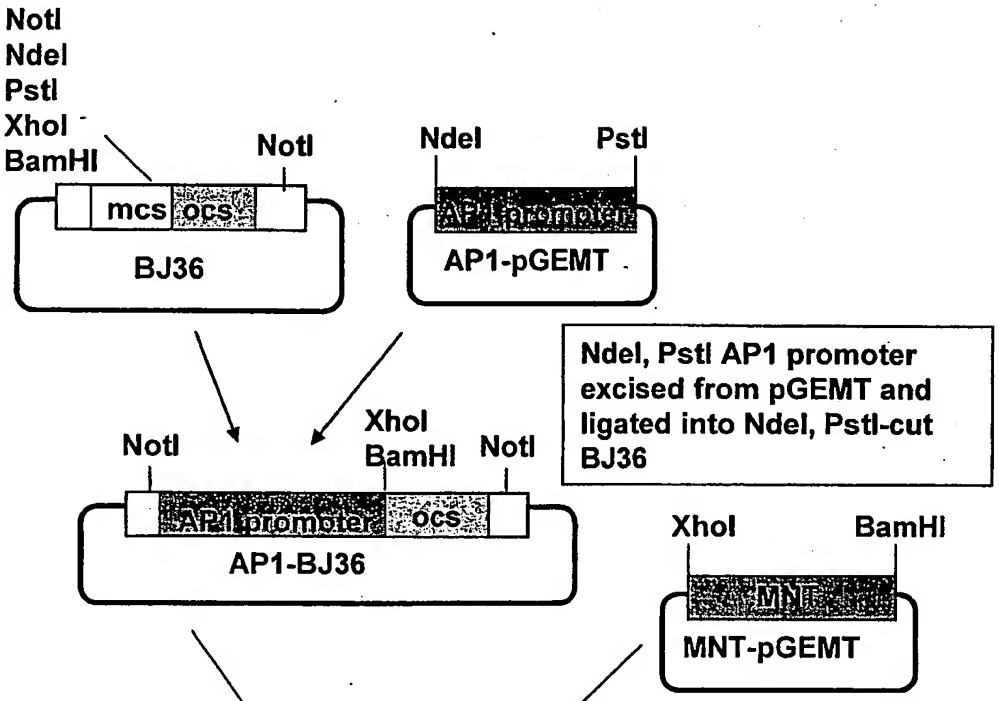


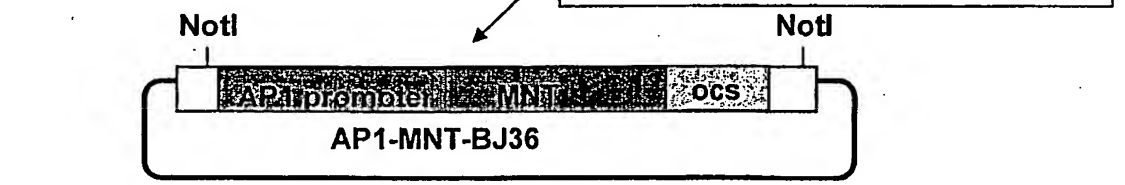
Figure 23

Cloning strategy, Example 15

Example 15a



Example 15b



Example 15c

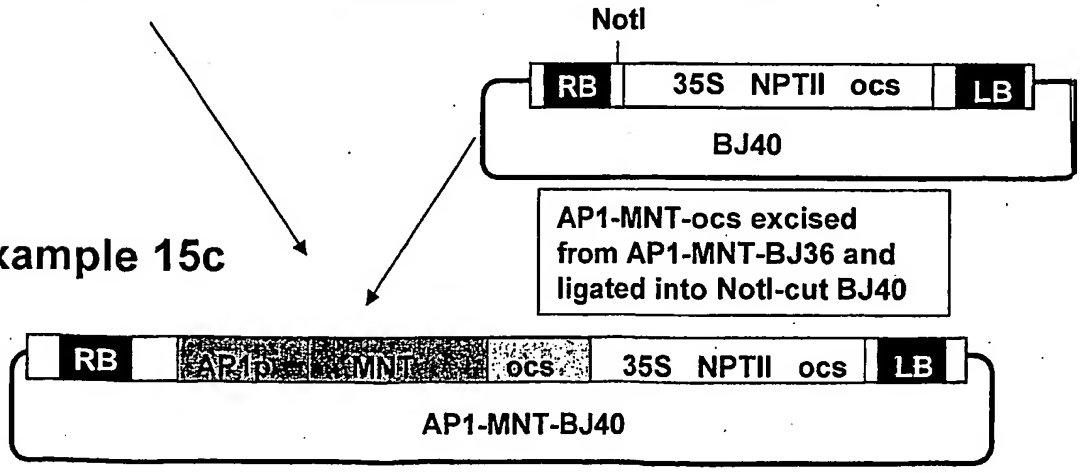
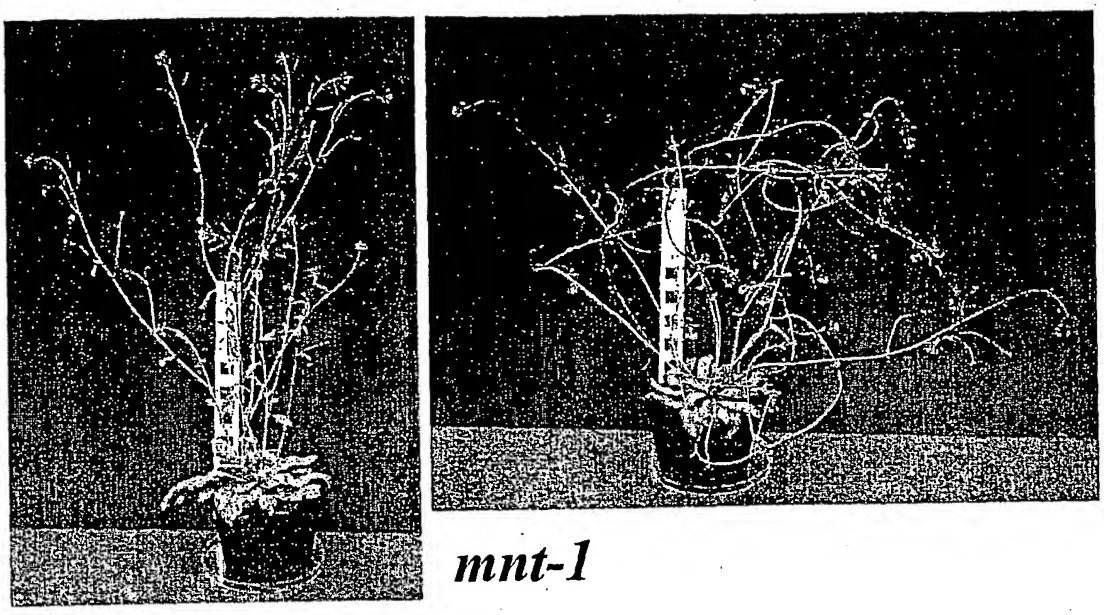


Figure 24

24A Wild-type vs *mnt-1* plants

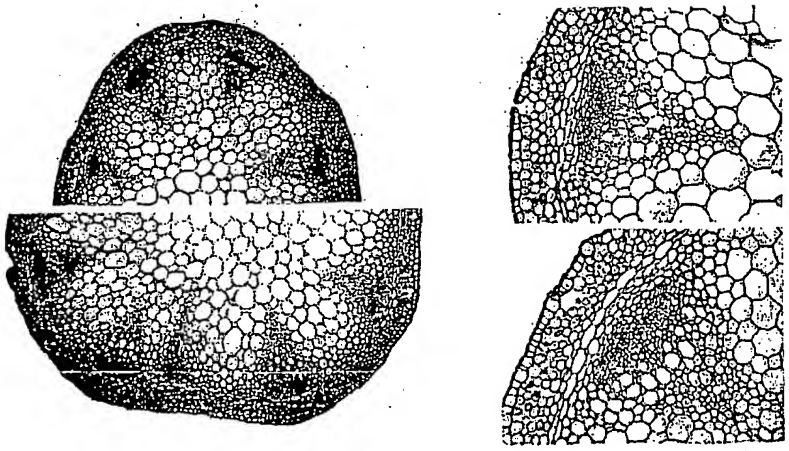


mnt-1

w.t.

24B Wild-type vs *mnt-1* stems, transverse sections

w.t.

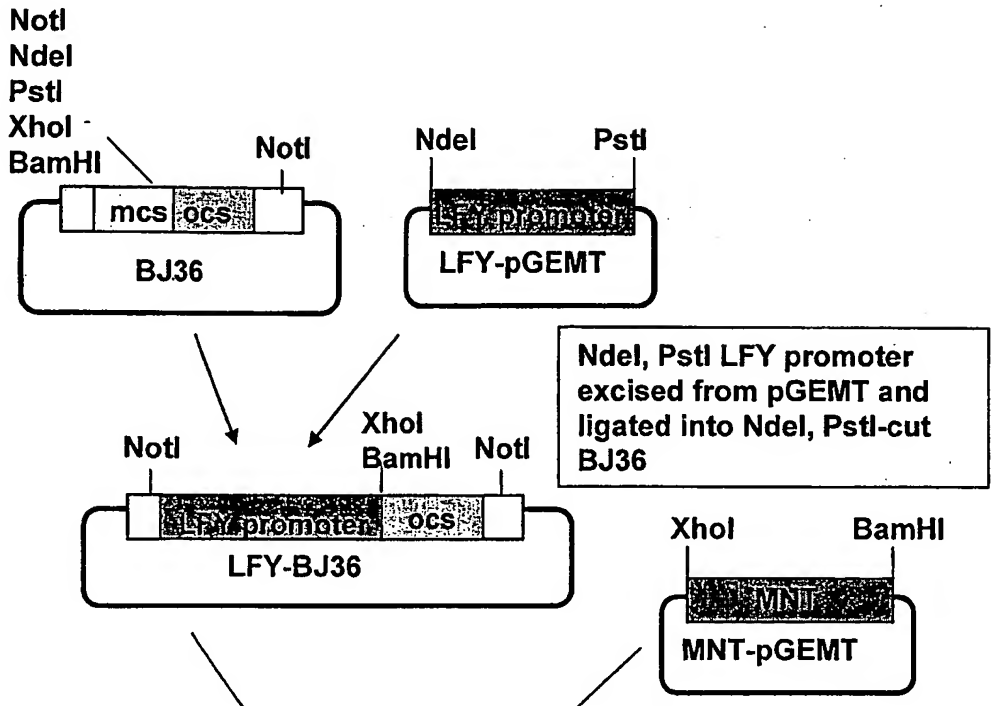


mnt-1

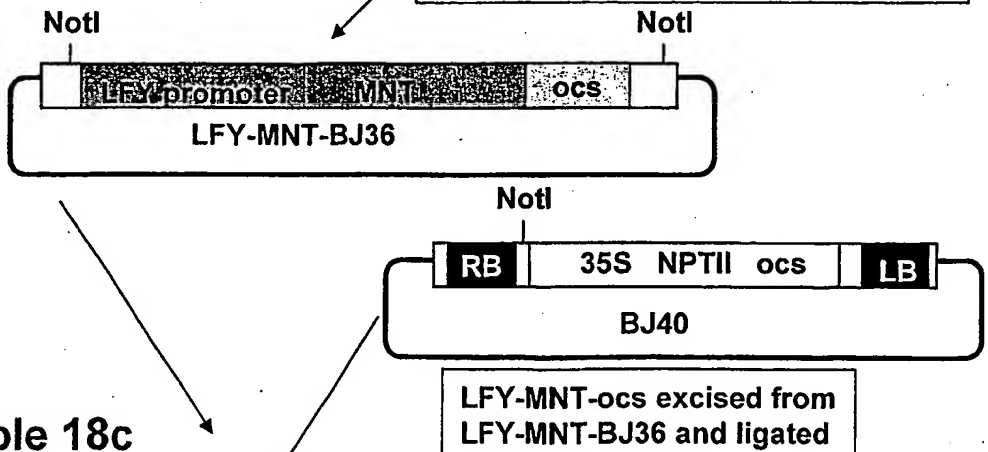
Figure 25

Cloning strategy, Example 18

Example 18a



Example 18b



Example 18c